

NORBERT W.
BISCHOFBERGER, Ph.D.

90-007448

07/652,978

B3



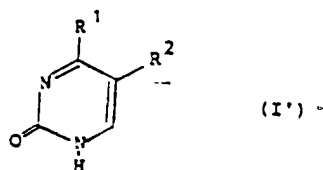
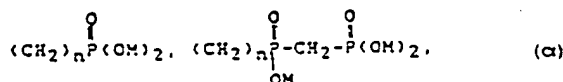
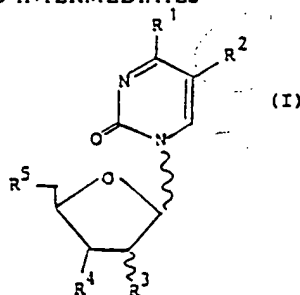
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁴ : C07H 19/06, 19/10, 19/24 A61K 31/70, C07D 239/47 C07D 239/54, 401/04, 403/04 C07D 405/04, 409/04, 421/04</p>	<p>A1</p>	<p>(11) International Publication Number: WO 89/12061 ✓ (43) International Publication Date: 14 December 1989 (14.12.89)</p>
<p>(21) International Application Number: PCT/SE89/00322 (22) International Filing Date: 7 June 1989 (07.06.89) (30) Priority data: 8802173-8 10 June 1988 (10.06.88) SE (71) Applicant (for all designated States except US): MEDIVIR AB [SE/SE]; Statens Bakteriologiska Laboratorium, Lundagatan 2, S-105 21 Stockholm (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): JOHANSSON, K. Nils, Gunnar [SE/SE]; Bäverstigen 19, S-150 23 Enhörna (SE). MALMBERG, Hans, C. G. [SE/SE]; Östastigen 49, S-151 52 Södertälje (SE). NOREEN, Rolf [SE/SE]; Kämpvågen 47, S-151 54 Södertälje (SE). SAHLBERG, S. Christer [SE/SE]; Målarhöjdsvägen 52, S-126 57 Hägersten (SE). SOHN, Daniel, D. [SE/SE];</p>		<p>Klinkervägen 11, S-151 55 Södertälje (SE). GRONOWITZ, Sulo [SE/SE]; Gerdagatan 16, S-223 62 Lund (SE). (74) Agents: LARFELDT, Helene et al.; Bergenstråhle & Lindvall AB, Sankt Paulsgatan 1, S-116 47 Stockholm (SE). (81) Designated States: AU, DK, FI, HU, JP, KR, NO, US. Published With international search report. NEW 1-RIBOFURANOSYL-5-HETEROCYCLYL OR ARYL-PYRIMIDIN-2-ONE DERIVS. USEFUL AS ANTIVIRAL AGENTS, ESP. AGAINST HIV, HEPATITIS B AND HERPES, AND NEW 1-UNSUBST. PRECURSORS</p>

(54) Title: PYRIMIDINE NUCLEOSIDES AND INTERMEDIATES



(57) Abstract

Compounds of formula (I), wherein R¹ is OH, NH₂; R² is a heteroaromatic or aromatic substituent as defined in claim 1; R³ is H, OH, F, OCH₃; R⁴ is H, F, OH or an ether or ester residue thereof, OCH₃, CN, C=CH, N₃; R⁵ is OH or an ether or ester residue thereof including mono-, di- and triphosphate esters; (α), wherein n is 0 or 1 and M is hydrogen or a pharmaceutically acceptable counterion such as sodium, potassium, ammonium or alkylammonium; and pharmaceutically acceptable salts thereof; and pharmaceutical compositions comprising said compounds can be used for therapeutic and/or prophylactic treatment of virus infections such as AIDS. Compounds of formula (I'), wherein R¹ and R² are as defined above, are new precursor compounds.

90007448

Pyrimidine nucleosides and intermediates.

Field of the invention

The present invention relates to novel chemical compounds and pharmaceutically acceptable salts thereof which can be used in therapy for therapeutic and prophylactic treatment of the acquired immuno deficiency syndrome (AIDS) and infections caused by viruses requiring reverse transcriptase for replication, such as human immuno deficiency viruses and hepatitis B virus, and also for treatment of other virus diseases, such as those of herpes viruses, diseases which include both common infections and neoplastic diseases, i.e. cancer. The invention also relates to novel precursor compounds constituting a further aspect of the invention.

Background of the invention

The effects of viruses on bodily functions is the end result of changes occurring at the cellular and subcellular levels. The pathogenic changes at the cellular level are different for different combinations of viruses and host cells. While some viruses cause a general destruction (killing) of certain cells, other may transform cells into a neoplastic state.

Important common viral infections are herpes dermatitis (including herpes labialis), herpes keratitis, herpes genitalis, herpes zoster, herpes encephalitis, infectious mononucleosis and cytomegalovirus infections all of which are caused by viruses belonging to the herpes virus group. Other important viral diseases are influenza A and B which are caused by influenza A and B virus respectively. Another important common viral disease is viral hepatitis and especially hepatitis B virus infections are widely spread. Effective and selective antiviral agents are needed for treatment of these diseases as well as for other diseases caused by viruses.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	IL	Israel
AU	Australia	FR	France	IN	India
BE	Belgium	GB	Great Britain	JP	Japan
BF	Burkina Faso	CA	Canada	KE	Kenya
BG	Bulgaria	CH	Switzerland	KG	Kazakhstan
BJ	Benin	CL	Chile	KR	South Korea
BR	Brazil	CM	Cameroon	LA	Laos
BU	Burundi	CO	Colombia	LT	Lithuania
CA	Canada	CY	Cyprus	LU	Luxembourg
CC	Cocos (Keeling) Islands	DE	Germany	MC	Monaco
CD	Congo	DK	Denmark	MD	Moldova
CE	Czech Republic	EE	Estonia		
CF	Central African Republic	EG	Egypt		
CG	Congo	ES	Spain		
CH	Switzerland				
CI	Ivory Coast				
CM	Cameroon				
CN	China				
CO	Colombia				
CR	Costa Rica				
CU	Cuba				
CZ	Czech Republic				
DE	Germany				
DK	Denmark				
DM	Dominican Republic				
DO	Dominican Republic				
DZ	Algeria				
EC	Ecuador				
EE	Estonia				
EG	Egypt				
EH	Western Sahara				
ES	Spain				
ET	Ethiopia				
FI	Finland				
FJ	Fiji				
FR	France				
GA	Gabon				
GB	Great Britain				
GD	Grenada				
GE	Georgia				
GF	French Guiana				
GG	Guernsey				
GH	Ghana				
GI	Guinea-Bissau				
GL	Greenland				
GM	Gambia				
GN	Guinea				
GP	Guadeloupe				
GQ	Equatorial Guinea				
GR	Greece				
GS	South Georgia and the South Sandwich Islands				
GT	Guatemala				
GU	Guam				
GW	Guinea-Bissau				
GX	French Guiana				
GY	Guyana				
HA	Haiti				
HB	Hong Kong				
HC	Honduras				
HD	Heard Island and McDonald Islands				
HE	Holsten				
HF	Hong Kong				
HG	Hong Kong				
HH	Hong Kong				
HI	Hong Kong				
HJ	Hong Kong				
HK	Hong Kong				
HL	Hong Kong				
HM	Hong Kong				
HN	Honduras				
HO	Hong Kong				
HP	Hong Kong				
HQ	Hong Kong				
HR	Croatia				
HS	Hong Kong				
HT	Haiti				
HU	Hungary				
IV	Ivory Coast				
IZ	Iceland				
JA	Japan				
JB	Japan				
JC	Japan				
JD	Japan				
JE	Jersey				
JF	Japan				
JG	Japan				
JH	Japan				
JI	Japan				
JK	Japan				
JL	Japan				
JM	Japan				
JN	Japan				
JO	Jordan				
JP	Japan				
JQ	Japan				
JR	Japan				
JS	Japan				
JT	Japan				
JU	Japan				
JV	Japan				
JW	Japan				
JX	Japan				
JY	Japan				
JZ	Japan				
KA	Kazakhstan				
KB	Kazakhstan				
KC	Kazakhstan				
KD	Kazakhstan				
KE	Kenya				
KF	Kazakhstan				
KG	Kazakhstan				
KH	Kazakhstan				
KI	Kazakhstan				
KJ	Kazakhstan				
KK	Kazakhstan				
KL	Kazakhstan				
KM	Kazakhstan				
KN	Kazakhstan				
KO	Kazakhstan				
KP	Kazakhstan				
KQ	Kazakhstan				
KR	South Korea				
KS	Kazakhstan				
KT	Kazakhstan				
KU	Kazakhstan				
KV	Kazakhstan				
KW	Kazakhstan				
KX	Kazakhstan				
KY	Kazakhstan				
KZ	Kazakhstan				
LA	Laos				
LB	Lebanon				
LC	Liechtenstein				
LD	Laos				
LE	Lebanon				
LF	Laos				
LG	Laos				
LH	Laos				
LI	Liechtenstein				
LJ	Laos				
LK	Laos				
LM	Laos				
LN	Laos				
LO	Laos				
LP	Laos				
LQ	Laos				
LR	Laos				
LS	Laos				
LT	Lithuania				
LU	Luxembourg				
LV	Latvia				
LY	Libya				
MA	Malaysia				
MB	Malawi				
MC	Monaco				
MD	Moldova				
ME	Montenegro				
MF	Malawi				
MG	Madagascar				
MH	Marshall Islands				
MI	Malawi				
MJ	Malawi				
MK	Malawi				
ML	Malawi				
MM	Malawi				
MN	Malawi				
MO	Malawi				
MP	Malawi				
MQ	Malawi				
MR	Malawi				
MS	Malawi				
MT	Malawi				
MU	Mauritius				
MV	Malawi				
MW	Malawi				
MX	Mexico				
MY	Malawi				
MZ	Malawi				
NA	Namibia				
NB	Namibia				
NC	Namibia				
ND	Namibia				
NE	Niger				
NF	Norfolk Island				
NG	Nigeria				
NH	Norway				
NI	Nicaragua				
NJ	Norway				
NK	Norway				
NL	Netherlands				
NM	Norway				
NN	Norway				
NO	Norway				
NP	Nepal				
NQ	Norway				
NR	Norway				
NS	Norway				
NT	Norway				
NU	Norway				
NV	Norway				
NW	Norway				
NY	Norway				
NZ	New Zealand				
OA	Other				
OB	Other				
OC	Other				
OD	Other				
OE	Other				
OF	Other				
OG	Other				
OH	Other				
OI	Other				
OJ	Other				
OK	Other				
OL	Other				
OM	Other				
ON	Other				
OO	Other				
OP	Other				
OQ	Other				
OR	Other				
OS	Other				
OT	Other				
OU	Other				
OV	Other				
OW	Other				
OX	Other				
OY	Other				
OZ	Other				
PA	Pakistan				
PB	Pakistan				
PC	Pakistan				
PD	Pakistan				
PE	Peru				
PF	Pakistan				
PG	Pakistan				
PH	Philippines				
PI	Pakistan				
PJ	Pakistan				
PK	Pakistan				
PL	Poland				
PM	Pakistan				
PN	Pakistan				
PO	Pakistan				
PP	Pakistan				
PQ	Pakistan				
PR	Pakistan				
PS	Pakistan				
PT	Portugal				
PV	Pakistan				
PW	Pakistan				
PX	Pakistan				
PY	Paraguay				
PZ	Pakistan				
QA	Qatar				
QB	Qatar				
QC	Qatar				
QD	Qatar				
QE	Qatar				
QF	Qatar				
QG	Qatar				
QH	Qatar				
QI	Qatar				
QJ	Qatar				
QK	Qatar				
QL	Qatar				
QM	Qatar				
QN	Qatar				
QO	Qatar				
QP	Qatar				
QQ	Qatar				
QR	Qatar				
QS	Qatar				
QT	Qatar				
QU	Qatar				
QV	Qatar				
QW	Qatar				
QX	Qatar				
QY	Qatar				
QZ	Qatar				
RA	Romania				
RB	Romania				
RC	Romania				
RD	Romania				
RE	Reunion				
RF	Romania				
RG	Romania				
RH	Romania				
RI	Romania				
RJ	Romania				
RK	Romania				
RL	Romania				
RM	Romania				
RN	Romania				
RO	Romania				
RP	Romania				
RQ	Romania				
RR	Romania				
RS	Romania				
RT	Romania				
RU	Romania				
RV	Romania				
RW	Romania				
RX	Romania				
RY	Romania				
RZ	Romania				
SA	South Africa				
SB	South Africa				
SC	South Africa				
SD	South Africa				
SE	Sweden				
SF	South Africa				
SG	Singapore				
SH	South Africa				
SI	Slovenia				
SJ	South Africa				
SK	South Africa				
SL	Sierra Leone				
SM	San Marino				
SN	Senegal				
SO	South Africa				
SP	Spain				
SQ	South Africa				
SR	South Africa				
SS	South Africa				
ST	St. Helena				
SV	Sweden				

Several different viruses of both DNA and RNA type have been shown to cause tumors in animals. The effect of carcinogenic chemicals can on animals result in activation of latent tumor viruses. It is possible that tumor viruses are involved in human tumors. The most likely human cases known today are leukemias, sarcomas, breast carcinomas, Burkitt lymphomas, nasopharyngeal carcinomas and cervical cancers where RNA tumor viruses and herpes viruses are indicated and papillomas where papilloma viruses are involved. This makes the search for selective inhibitors of tumorigenic viruses and their functions an important undertaking in the efforts to treat cancer.

In the late seventies a new disease was reported, which subsequently was referred to as Acquired Immuno Deficiency Syndrome (AIDS). It is now generally accepted that a retrovirus referred to as HIV (Human Immunodeficiency Virus), formerly known as Human T-cell Lymphotropic Virus (HTLV-III) or Lymphadenopathy Associated Virus (LAV) plays an essential role in the etiology of AIDS. Different types of HIV have been found, such as HIV-1 and HIV-2 and more are likely to be isolated.

AIDS is characterized by a profound immunodeficiency due to low numbers of a subset of lymphocyte-T-helper cells, which are one target for HIV infection. The profound immunodeficiency in AIDS patients makes these patients highly susceptible to a variety of opportunistic infections of bacterial, fungal, protozoal or viral etiology. The etiological agents among viral opportunistic infections are often found in the herpes virus group, i.e. herpes simplex virus (HSV), Varicella Zoster virus (VZV), Epstein-Barr virus (EBV) and, especially, cytomegalovirus (CMV). Other retroviruses affecting humans are HTLV-1 and II and examples of retroviruses affecting animals are feline leukemia virus and equine infectious anemia virus. Human diseases such as multiple sclerosis, psoriasis, tropical spastic paresis and Kawasaki disease have also been reported to be associated with retrovirus infections.

4416

90007448

Hepatitis B virus infections cause severe disease such as acute hepatitis, chronic hepatitis, fulminant hepatitis in a considerable number of persons. It is estimated that there are 200 million patients with chronic hepatitis B infection in the world. A considerable number of the chronic cases progress to liver cirrhosis and liver tumours. In some cases the hepatitis infections also take a rapid and severe course as in fulminant B hepatitis with about 90% mortality. At present there is no known effective treatment against hepatitis B infections. The replication of hepatitis B virus is similar to that of retroviruses and it contains the same essential viral reverse transcriptase activity.

General outline of the invention

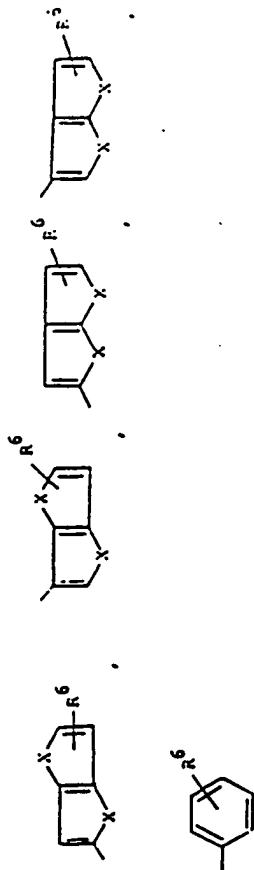
A great number of nucleoside analogues exhibit several antimetabolic activities. They do so by substituting for or competing with the naturally occurring nucleosides. Recently some nucleoside analogues have been described, which inhibit in cell culture the multiplication of human immunodeficiency virus (HIV, also called HTLV-III, LAV) the causative agent of AIDS and AIDS-related complex (ARC).

We have now found that activities for inhibition of HIV and/or herpes multiplication are exhibited by nucleoside analogues, in which the pyrimidine bases are substituted in the 5-position by a heteroaromatic, or aromatic substituent. The nucleoside analogues may be either alpha- or beta-anomers.

4417

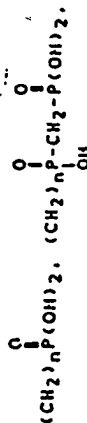
90007448

SUBMITTED TO THE



wherein X is O, S, N-R⁷, Se;
 R⁶ is H, straight or branched C₁-10 alkyl, F, Cl, Br, I,
 X-R⁷, -CH=CH-R⁷, -C≡C-R⁷, CO₂R⁷, CH₂X-R⁷;
 R⁷ is H, straight or branched C₁-5 alkyl, phenyl;

R³: H, OH, F, OCH₃;
 R⁴: H, F, OH or an ether or ester residue thereof, OCH₃, CN,
 C≡CH, N₃;
 R⁵: OH or an ether or ester residue thereof;

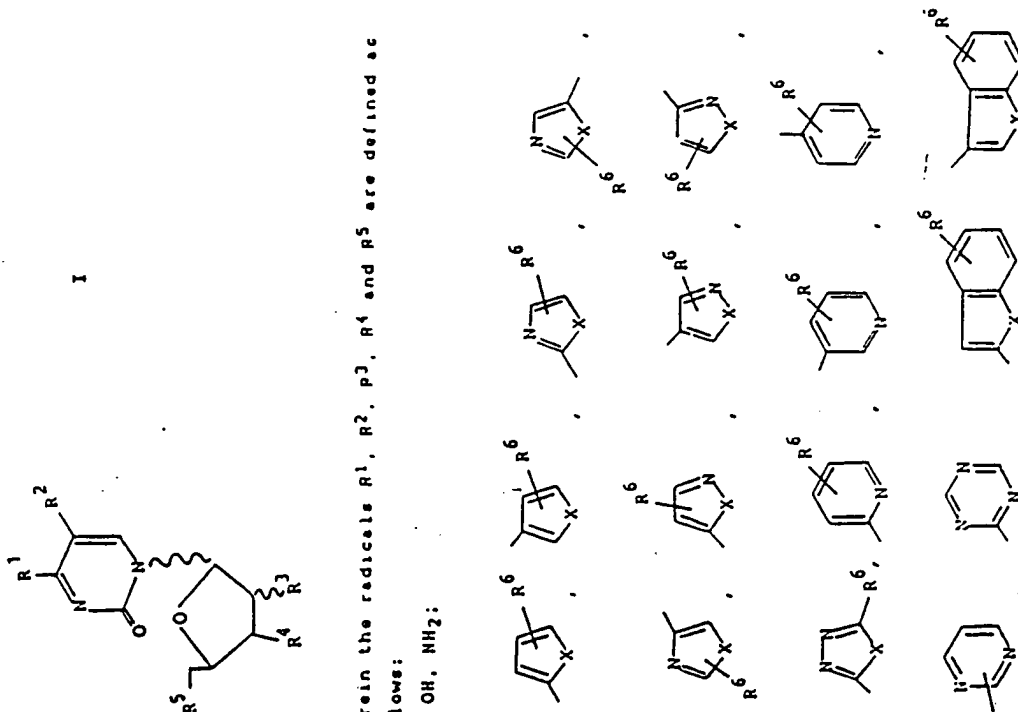


wherein n is 0 or 1 and H is hydrogen or a pharmaceutically acceptable counterion such as sodium, potassium, ammonium or alkylammonium; and pharmaceutically acceptable salts thereof. Said compounds have been found to inhibit the multiplication of human immunodeficiency virus (HIV).

The invention consequently also refers to the compounds of the formula I for use in therapy. The compounds of the formula I are useful as a therapeutic and/or prophylactic agents in the control and treatment of HIV virus infections in man. In a more general aspect, the compounds of the formula I are useful as therapeutic and/or prophylactic agents in the control and treatment of infections caused by retroviruses and hepatitis B virus in mammals and man.

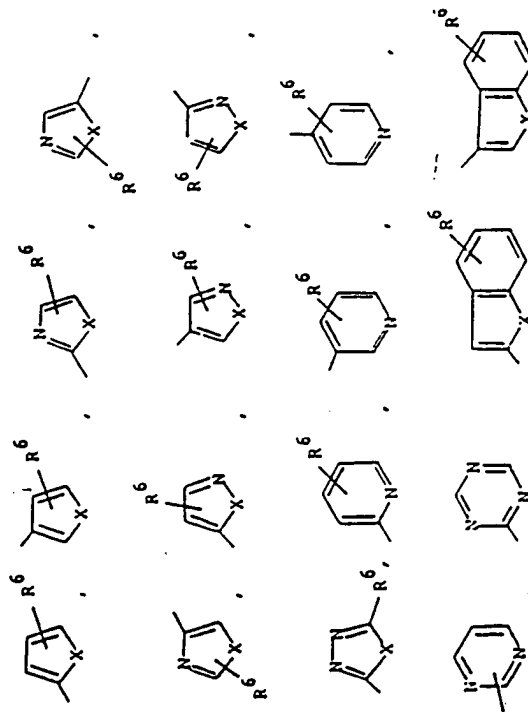
Disclosure of the invention

The present invention relates to new compounds of the formula I:



wherein the radicals R¹, R², R³, R⁴ and R⁵ are defined as follows:

R¹: OH, NH₂;
 R²:



All retroviruses, including HIV, require the enzyme reverse transcriptase in their natural cycle of replication.

Hepatitis B virus (HBV) is a DNA virus with a unique circular double-stranded DNA genome which is partly single-stranded. It contains a specific DNA polymerase required for viral replication. This DNA polymerase also acts as a reverse transcriptase during the replication of HBV DNA via an RNA intermediate.

The compounds of the formula I inhibit the activity of reverse transcriptase of retroviruses including HIV as well as the activity of DNA polymerase of hepatitis B virus.

Another important area of use for the compounds of the formula I is in the treatment of herpes virus infections. Among the herpes viruses may be mentioned Herpes simplex type 1 and 2, varicella (Herpes zoster), virus causing infectious mononucleosis (i.e. Epstein-Barr virus), cytomegalovirus and human herpes virus type 6. Important diseases caused by herpes viruses are herpes dermatitis (including herpes labialis), herpes genitalis, herpes keratitis, herpes encephalitis and herpes zoster.

Another possible area of use for the compounds of the present invention is in the treatment of cancer and tumors, particularly those caused by viruses. This effect may be obtained in different ways, i.e. by inhibiting the transformation of virus-infected cells to a neoplastic state, by inhibiting the spread of viruses from transformed cells to other normal cells and by arresting the growth of virus-transformed cells.

The invention furthermore provides:

A pharmaceutical composition comprising a compound of the formula I as an active ingredient and a pharmaceutically acceptable carrier, including liposomes; and

90007448

4420

A method for therapeutic and/or prophylactic treatment of virus infections in an animal or human host in need of treatment: comprising administering an effective amount of a compound of the formula I.

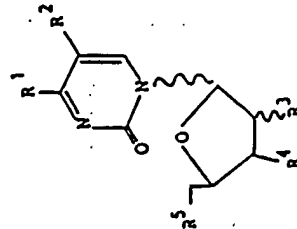
It is a preferred aspect of the invention to treat infections caused by herpes viruses or viruses requiring reverse transcriptase for replication, including human immuno deficiency viruses and hepatitis B virus.

The invention also relates to the use of a compound of the formula I for the manufacture of a medicament for therapeutic and/or prophylactic treatment of the acquired immuno deficiency syndrome and infections caused by viruses requiring reverse transcriptase for replication.

Preferably they can be used for the treatment of infections caused by HIV viruses or hepatitis B virus.

The nucleoside analogues of the invention are composed of a 5-substituted uracil or cytosine base and a sugar moiety which can for instance be ribose, 2'-deoxyribose, 2',3'-dideoxyribose, arabinose, or analogues thereof.

Preferred compounds of the formula I



90007448

4421

8

are those wherein

R² is 2-furyl, 2-thienyl, selenienyl, thiazolyl;

2-(1-alkyl)pyrrolyl or methoxyphenyl;

R³ is hydrogen, hydroxy or fluoro;

R⁴ is hydrogen, hydroxy, fluoro, cyano or azido; and

R⁵ is hydroxy, a mono-, di- or triphosphate thereof or

C

CH₂(OH)₂, wherein M is a pharmaceutically acceptable counterion.

Examples of especially preferred compounds are those of the formula I wherein:

R ¹	R ²	R ³	R ⁴	R ⁵
OH	2-furyl	H	OH	OH or triphosphate
OH	2-thienyl	H	OH	OH or triphosphate
OH	2-selenienyl	H	OH	OH or triphosphate
OH	2-thiazolyl	H	OH	OH or triphosphate
OH	2-furyl	OH	OH	OH or triphosphate
OH	2-thienyl	OH	OH	OH or triphosphate
OH	2-selenienyl	OH	OH	OH or triphosphate
OH	2-thiazolyl	OH	OH	OH or triphosphate
OH	2-furyl	OH	F	OH or triphosphate
OH	2-thienyl	OH	F	OH or triphosphate
OH	2-selenienyl	OH	F	OH or triphosphate
OH	2-thiazolyl	OH	F	OH or triphosphate
OH	2-furyl	OH	N ₃	OH or triphosphate
OH	2-thienyl	OH	N ₃	OH or triphosphate
OH	2-selenienyl	OH	N ₃	OH or triphosphate
OH	2-thiazolyl	OH	N ₃	OH or triphosphate
OH	2-furyl	H	H	OH or triphosphate
OH	2-thienyl	H	H	OH or triphosphate
OH	2-selenienyl	H	H	OH or triphosphate
OH	2-thiazolyl	H	H	OH or triphosphate

4422

90007448

4423

90007448

9

OH	2-furyl	H	F	OH or triphosphate
OH	2-thienyl	H	F	OH or triphosphate
OH	2-selenienyl	H	F	OH or triphosphate
OH	2-thiazolyl	H	F	OH or triphosphate
OH	2-furyl	H	N ₃	OH or triphosphate
OH	2-thienyl	H	N ₃	OH or triphosphate
OH	2-selenienyl	H	N ₃	OH or triphosphate
OH	2-thiazolyl	H	N ₃	OH or triphosphate
OH	2-furyl	H	OH	methylphosphonate
OH	2-thienyl	H	OH	methylphosphonate
OH	2-thiazolyl	H	OH	methylphosphonate
OH	2-furyl	H	H	methylphosphonate
OH	2-thienyl	H	H	methylphosphonate
OH	2-thiazolyl	H	H	methylphosphonate
OH	2-furyl	H	F	methylphosphonate
OH	2-thienyl	H	F	methylphosphonate
OH	2-thiazolyl	H	F	methylphosphonate
OH	2-furyl	H	N ₃	methylphosphonate
OH	2-thienyl	H	N ₃	methylphosphonate
OH	2-thiazolyl	H	N ₃	methylphosphonate
OH	2-furyl	OH	F	methylphosphonate
OH	2-thienyl	OH	F	methylphosphonate
OH	2-thiazolyl	OH	F	methylphosphonate

Esters and ethers of the nucleosides are also included in the invention. Examples of esters are mono-, di- and tri-phosphate esters, carboxylic esters, carbonate esters, carbamate esters and sulphonic esters. The acid part of the esters may have alkyl, aryl or arylalkyl chains, where the aryl functionalities are optionally substituted for example by alkoxy, amino, nitrile, alkyl or sulphonamido groups or by one or more halogen atoms. Examples of other types of derivatives of the nucleosides are alkyl or arylalkyl derivatives of the 5-hydroxyl group. The arylalkyl ether derivatives may be for example benzyl or tri-phenyl methyl and the aryl moiety may be optionally

substituted. Furthermore, it is understood that the examples of the pharmaceutically acceptable salts cited below also apply to the various esters or derivatives of the nucleosides of the invention.

in a compound of the formula I R⁵ as an ether residue can be defined as OR⁸, wherein R⁸ is C₁-6 alkyl, arylalkyl; optionally substituted with one or more alkoxy, amino, nitrile or sulphamido groups or one or more halogen atoms.

R⁶ and R⁵ as an ester residue can be derived from a carboxylic acid R⁹COOH, a carbonic acid R¹⁰OCOOH, a double ester of a carbonic acid R¹⁰CO₂CH(R¹¹)OCO₂H, a sulphonic acid R¹⁰SO₂OH, a carbamic acid R¹⁰NHCOOH or a phosphoric acid, wherein R⁹ is hydrogen, C₁-17 alkyl, alkoxyalkyl, arylalkyl or aryl, R¹⁰ is C₁-17, arylalkyl or aryl, R¹¹ is hydrogen or C₁-3 alkyl and said aryl and arylalkyl groups optionally can be substituted with one or more alkyl, alkoxy, amino, nitrile, sulphonamido groups or one or more halogen atoms.

Examples of pharmaceutically acceptable salts of the compounds of formula I include base salts, e.g. derived from an appropriate base, such as alkali metal (e.g. sodium, potassium, alkaline earth metal; e.g. magnesium) salts, ammonium and NX₄⁺ (wherein X is C₁-4 alkyl). Physiologically acceptable acid salts include salts of organic carboxylic acids such as acetic, lactic, gluconic, citric, tartaric, malic, malic, pantothenic, isethionic, oxalic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic, p-chlorobenzenesulphonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, hydroiodic, sulfuric, phosphoric and sulfamic acids.

Physiologically acceptable counterions M of the phosphonate groups include inorganic and organic counterions. Inorganic

90007448

counterions are (for example ammonium, sodium, potassium,

lithium, magnesium and calcium. Organic counterions are derived from non-toxic bases, such as primary, secondary and tertiary amines, including naturally occurring amines. Examples of such amines are diethylamine, triethylamine, isopropylamine, ethanolamine, morpholine, 2-diethylaminoethanol, glucosamine, N-methylglucamine, piperazine and dicyclohexylamine.

In clinical practice the pyrimidine derivatives of the formula I will normally be administered orally, by injection or by infusion in the form of a pharmaceutical preparation comprising the active ingredient in the form of the original compound or optionally in the form of a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier which may be a solid, semi-solid or liquid diluent or an ingestible capsule. The compound may also be used without carrier material. As examples of pharmaceutical preparations may be mentioned tablets, dragees, capsules, granulates, suspensions, elixirs, syrups, solutions, liposomes etc. Usually the active substance will comprise between 0.05 and 20% for preparations intended for injection and between 10 and 90% for preparations intended for oral administration.

In the treatment of patients suffering from retrovirus, especially HIV, or hepatitis B virus infections, it will be preferred to administer the compounds by any suitable route including the oral, parenteral, rectal, nasal, topical and vaginal route. The parenteral route includes subcutaneous, intramuscular, intravenous and sublingual administration. The topical route includes buccal and sublingual administration. The dosage at which the active ingredients are administered may vary within a wide range and will depend on various factors such as the severity of the infection, the age of the patient etc., and may have to be individually adjusted. As a possible range for

90007448

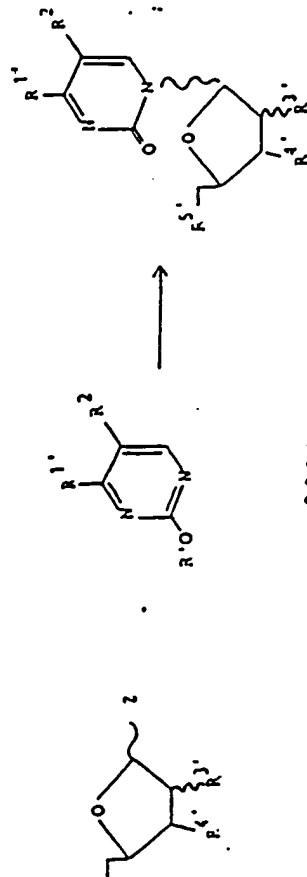
Particularly preferred types of interferon are α , β and γ and interferon inducers such as "Ampligen" (Ham Research).

Other combinations suitable for use according to the present invention include those wherein the second agent is, for example, interleukin 1, suramin, foscarnet or an ester thereof, fluorothymidine, HPA 23, inhibitors of HIV protease such as pepstatin, steroids, medications such as levamisole or thymosin to increase lymphocyte numbers and/or function as appropriate, or GM-CSF and other factors regulating cell functions.

Methods of preparation

The compounds of the invention may be prepared by one of the following general methods, constituting a further aspect of the invention.

A. Condensing a glycoside as comprised in formula 1 where the hydroxyl groups may be optionally protected to the N-1 position of a pyrimidine derivative, according to known methods described in the literature. Such methods are described for example in "Basic Principles in Nucleic Acid Chemistry", Vol. 1 (Academic Press, 1974, Ed. P.O.P.Te'o), in "Nucleoside Analogues, Chemistry, Biology and Medical Applications" (Pharma Press, 1979, Eds. R.T. Walker, E. De Clercq and F. Eckstein).



90007448

SUBSTITUTE SHEET

4427

the amount of the compounds of the invention or a physiologically acceptable salt thereof to be administered per day may be mentioned from about 10 mg to about 10 000 mg, preferentially 100-500 mg for intravenous administration and preferentially 100-3000 mg for oral administration.

Compounds of the formula 1 can cooperate synergistically or additively with a wide range of other therapeutic agents, thereby enhancing the therapeutic potential of both agents without adding the toxic effects, thus increasing the therapeutic ratio.

Therefore, a compound of formula 1 or a pharmaceutically acceptable derivative thereof can be used in combination therapy, wherein the two active agents are present in a ratio resulting in an optimal therapeutic ratio. This can be provided either by a synergistic effect against the viral infection and/or by a decrease in toxicity while maintaining a therapeutic effect which is additive or synergistic.

The optimal therapeutic ratio is observed when the two agents are present in a ratio of 500:1 to 1:500, preferably 100:1 to 1:100, particularly 20:1 to 1:20 and especially 10:1 to 1:10.

Said combinations may conveniently be administered together, for example, in a unitary pharmaceutical formulation, or separately for example as a combination of tablets and injections administered at the same time or at different times, in order to achieve the required therapeutic effect.

The compounds of the formula 1 are potentiated by interferons, other antiviral agents such as foscarnet, AZT, HIV protease inhibitors, immunomodulators, interferon inducers and growth factors.

90007448

4426

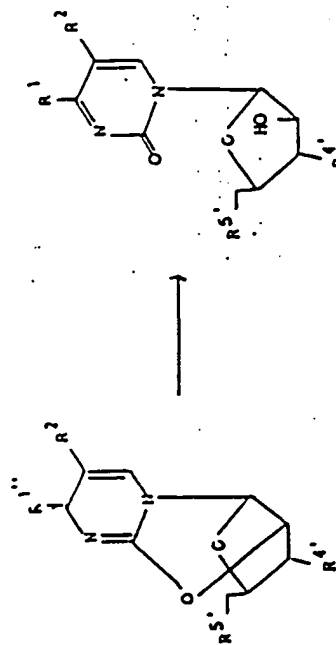
Examples of suitable derivatives of the reacting species are those wherein Z is Cl, Br, I, acyloxy or alkoxy; R' is an alkyl or silyl protecting group, such as C₂H₅ or (CH₃)₃Si; R¹ is R¹ as defined above, OC₂H₅, (CH₃)₃SiO, or H(COCH₃)Si(CH₃)₂; R² is as defined above; R³ and R⁴ is R³ and R⁴ respectively as defined above with the proviso that when R³ or R⁴ is OH said OH must be protected as O-acyl, O-benzoyl, O-benzyl or Q-silyl; (e.g. dimethyl, tert-butyldimethyl); and R⁵ is R⁵ as defined above or OR⁸ wherein R⁸ is as defined above or silyl (e.g. dimethyl, tert-butyldimethyl). After condensation the products may be hydrolyzed or converted by conventional methods, known to those skilled in the art, into compounds of the formula I.

The glycosides are known or may be prepared by suitable adaptations of known methods. The syntheses of a 2,3-dideoxy-3-fluoro-erythro-pentofuranoside for example, has been described by G.W.J. Fleet and J.C. Son in Tetrahedron Letters 10 (1987) pp 3615-3618. The other 3- substituents may be introduced by methods analogous to those described above and described by N.B. Dyathina and A.V. Azharyev in Syntheses 1984 pp 961-963. The arabinosylglycosides may be prepared by similar methods.

B. The β-anomers of the arabinosyl-pyrimidine nucleoside analogues may be prepared by hydrolysis of the corresponding 2,2'-anhydro nucleoside analogues.

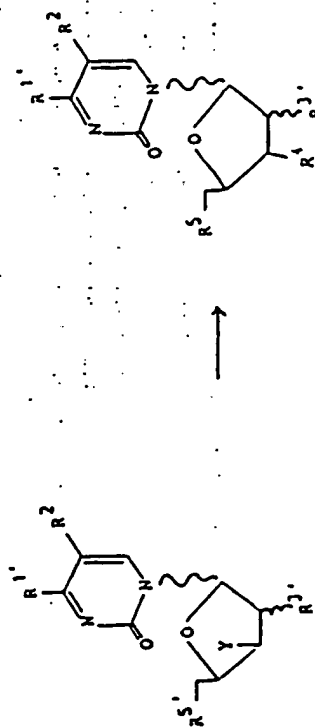
4428

90007448



wherein R¹ is O or NH and R¹, R², R⁴ and R⁵ are as defined above. The hydrolysis may be performed by conventional methods, described in the literature and known to those skilled in the art. It may for example be performed by treating the 2,2'-anhydronucleosides with an aqueous acid.

C. The halogeno, OCH₃, H₃, CN and C≡CH substituents in the 3'-position of the glycon moiety may be introduced by substitution of a hydroxyl group or a suitably derivatized hydroxyl group



4429

90007448

SUBSTITUTE SHEET

16

wherein Y is OH or a functionality that will be leaving in the substitution reaction such as for example CF₃SO₃ and R¹, R², R³, R⁴ and R⁵ are as defined above.

The following examples will further illustrate the invention:

Example 1 1-(2-Deoxy-3,5-di-O-p-toluy)-alpha-D-ribofuranosyl)-5-(furyl)uracil (VSB 0051) and

Example 2 1-(2-Deoxy-3,5-di-O-p-toluy)-beta-D-ribofuranosyl)-5-(furyl)uracil (VSB 0052)

5-(2-Furyl)uracil (150 mg, 0.84 mmol) in hexamethyldisilazane (10 ml) was heated at reflux for 5 hours together with chlorotrimethylsilane (10 drops) and ammonium sulfate (a few mg). The solution was filtered and evaporated in vacuo to dryness to give bis-trimethylsilylated 5-(2-furyl)uracil (240 mg) as a crude product. This crude product was dissolved in acetonitrile (15 ml, dried over molecular sieves) and added to a solution of 2-deoxy-3,5-di-O-p-toluy)-D-erythro-pentoseyl chloride (331 mg, 0.85 mmoles; prepared according to C.C.Bhat in Synthetic Procedures in Nucleic Acid Chemistry, Vol. 1, p. 521, Interscience Publ. 1968; W.W. Zorbach and R.S. Tipson eds.) in dry 1,2-dichloroethane (25 ml). The mixture was stirred at ambient temperature over night after which it was filtered. The solution was washed with an aqueous, saturated solution of sodium bicarbonate (50 ml) and water (50 ml), dried over sodium sulfate, concentrated to a volume of about 25 ml and refrigerated. The precipitate was filtered and recrystallized from 1,2-dichloroethane to give pure beta-anomer (0.70 g). The remaining combined solutions were evaporated and the residue was separated on a column of silica eluted with chloroform-ethyl acetate 5-1, to give the pure alpha-anomer, VSA 120, (0.51 g, m.p. 201-30°C) and the pure beta-anomer, VSA 125, (total combined yield 0.86 g, m.p. 217-30°C). Thin layer chromatography (silica, chloroform-ethyl acetate 5-1) R_f: alpha 0.23; beta 0.30.

Example 3 1-(2-Deoxy-3,5-di-O-p-toluy)-alpha-D-ribofuranosyl)-5-(2-thienyl)uracil (VSA 128) and

17

Example 4 1-(2-Deoxy-3,5-di-O-p-toluy)-beta-D-ribofuranosyl)-5-(2-thienyl)uracil (VSA 129)

5-(2-Thienyl)uracil (0.97 g, 5 mmol) in hexamethyldisilazane (10 ml) was heated at reflux for about 2.5 hours together with chlorotrimethylsilane (10 drops) and ammonium sulfate (a few mg). The solution was filtered and evaporated in vacuo to dryness to give bis-trimethylsilylated 5-(2-thienyl)uracil, which was dissolved in 1,2-dichloroethane (25 ml, dried over molecular sieves) and added to a solution of 2-deoxy-3,5-di-O-p-toluy)-D-erythro-pentoseyl chloride (1.55 g, 4 mmoles; prepared according to C.C. Bhat in Synthetic Procedures in Nucleic Acid Chemistry, Vol. 1, p. 521, Interscience Publ. 1968; W.W. Zorbach and R.S. Tipson eds.) in dry 1,2-dichloroethane (25 ml).

Molecular sieves (2 g, 4A) was added and the mixture was stirred at ambient temperature over night after which it was filtered. The solution was washed with an aqueous, saturated solution of sodium bicarbonate (50 ml) and water (50 ml), dried over sodium sulfate, concentrated to a volume of about 25 ml and refrigerated. The precipitate was filtered and recrystallized from 1,2-dichloroethane to give pure beta-anomer (0.70 g). The remaining combined solutions were evaporated and the residue was separated on a column of silica eluted with chloroform-ethyl acetate 5-1, to give the pure alpha-anomer, VSA 120, (0.51 g, m.p. 201-30°C) and the pure beta-anomer, VSA 125, (total combined yield 0.86 g, m.p. 217-30°C). Thin layer chromatography (silica, chloroform-ethyl acetate 5-1) R_f: alpha 0.23; beta 0.30.

Analysis for C₂₃H₂₈N₂O₇S; calculated (found) %:

alpha: C 63.72 (63.5); H 4.80 (4.8); N 5.13 (5.0);

beta: C 63.72 (63.2); H 4.80 (4.8); N 5.13 (5.1).

Analogous to examples 1 and 2, table 1 lists some further examples which were characterized as shown in table 2.

4430

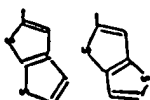
90007448

4431

90007448

Table 1. Examples of 1-(2-deoxy-3,5-di-O-P-toluoyl- α /beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	α /beta	R ²
1	α /beta	2-furyl
2	beta	2-furyl
3	α /beta	2-thienyl
4	beta	2-thienyl
5	α /beta	3-furyl
6	α /beta	3-thienyl
7	beta	3-thienyl
8	α /beta	2-selenienyl
9	beta	2-selenienyl
10	α /beta	3-selenienyl
11	beta	3-selenienyl
12	α /beta	2-pyridyl
13	α /beta	3-pyridyl
14	α /beta	4-pyridyl
15	α /beta	2-(5-methylthienyl)
16	beta	2-(5-methylthienyl)
17	α /beta	2-(5-hexylthienyl)
18	beta	2-(5-hexylthienyl)
55	α /beta	2-trans-tloften
56	beta	2-trans-tloften
57	α /beta	2-cis-tloften
58	beta	2-cis-tloften
59	α /beta	2-methoxyphenyl
60	beta	2-methoxyphenyl
61	α /beta	3-methoxyphenyl



4432

90007448

Table 2. Data for 1-(2-deoxy-3,5-di-O-P-toluoyl- α /beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	¹³ C NMR (CDCl ₃) ^δ					¹ H NMR (CDCl ₃) ^δ	m.p. °C	Thin layer chromatography R _F	
	1'	2'	3'	4'	5'				
1	88.1	39.4	74.9	85.8	64.3	6.44 d		0.37	a
2	85.8	38.7	75.1	83.3	64.7	6.53 t		0.50	a
3	88.0	39.2	74.6	85.7	64.0	6.41 d	201-3	0.23	b
4	85.9	38.8	75.0	83.5	64.4	6.48 t	217-9	0.30	b
5	88.1	39.3	74.8	85.8	64.2	6.33 d (J 3.5 Hz)	179-81	0.20	b
6									
7									
8							182-4	0.22	b
9							214-6	0.33	b
10									
11									
12	88.1	39.5	74.5	85.5	64.0	6.11 d (J 3.1 Hz)		0.12	a
13	88.1	39.2	74.8	85.8	64.1	6.41 d			
14						6.44		0.10	c
15	88.0	39.2	74.8	85.7	64.2	6.41 d (J 3.3 Hz)		0.17	d
16	85.7	38.6	75.0	83.2	64.4	6.46 t		0.26	d
17	88.0	39.3	74.8	85.8	64.2	6.40 d (J 2.8 Hz)		0.22	d
18	85.7	38.5	75.0	83.2	64.4	6.49 t (J 2.0 Hz)		0.34	d

a) CH₂Cl₂-EtOAc 5-1; b) CHCl₃-EtOAc 5-1; c) CHCl₃-EtOAc 4-1;
d) CHCl₃-EtOAc 9-1.

4433

90007448

Table 2 Data for 1-(2-deoxy-3,5-di-O-P-toluoyl-alpha/beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	¹³ C NMR (CDCl ₃) ^δ					¹ H NMR (CDCl ₃) ^δ	m.p. °C	Thin layer chromatography R _f
	1'	2'	3'	4'	5'	1'		
55							98-101	0.57 a
56							213-217	0.70 a
57							111-115	0.41 a
58							215-218	0.54 a
59							88-90	0.23 a
60							196-198	0.55 a
61							167-169	0.28 a

20

Example 19 1-(2-Deoxy-3)Pha-D-ribofuranosyl)-5-(2-(furyl)uracil)
(VSB-0072)

VSB 005 (62 mg, 0.117 mmol) was suspended in methanol (15 ml, dried over molecular sieves) and sodium methoxide in methanol (1.2 ml, 0.2 M) was added. The mixture was stirred at ambient temperature under an atmosphere of nitrogen for 24 hours, after which an ion exchanger, Dowex 50 Wx8 H⁺, was added. The solution was filtered and the solvent evaporated in vacuo. The residue was purified on a column of silica eluted with ethyl acetate-ethanol 9-1, to give 1-(2-deoxy-β-D-ribofuranosyl)-5-(2-(furyl)uracil) 32 mg (93%). Thin layer chromatography (silica, ethyl acetate-ethanol 18-1) R_f: 0.42.

Example 20 1-(2-Deoxy-beta-D-ribofuranosyl)-5-(2-(furyl)uracil)
(VSB-004)

VSB 006 (20 mg, 0.035 mmol) was hydrolyzed with sodium methoxide as described for VSB 005. After completion of reaction, the dry residue of the crude product was triturated with hexane and purified on silica to give 1-(2-deoxy-β-D-ribofuranosyl)-5-(2-(furyl)uracil). Thin layer chromatography (silica, ethyl acetate-ethanol 18-1) R_f: 0.47.

Example 21 1-(2-Deoxy-3)Pha-D-ribofuranosyl)-5-(2-(thienyl)uracil)
(VSA-1342)

VSA 128 (0.35 g, 0.64 mmol) was dissolved in methanol (50 ml) and sodium methoxide in methanol (5 ml, 0.2 M) was added. The solution was stirred at ambient temperature over night, after which it was neutralized with Dowex 50Wx8 H⁺. The solution was filtered, evaporated in vacuo and the residue was triturated with diethyl ether to give a solid residue 1-(2-deoxy-β-D-ribofuranosyl)-5-(2-(thienyl)uracil). Thin layer chromatography (silica, chloroform-methanol 85-15) R_f: 0.44. Analyte for

21

4434

90007448

4435

90007448

$C_{13}H_{14}N_2O_5$, calculated (found) %: C 50.31 (50.3); H 4.35 (4.5); N 9.07 (8.8).

Example 22 1-(2-Deoxy-beta-D-ribofuranosyl)-5-(2-thienyl)uracil (VSA 133)

The title compound was prepared from VSA 125 (0.55 g, 1 mmol) in the same way as has been described for the corresponding alpha-anomer VSA 134. Thin layer chromatography for VSA 133 (silica, chloroform-methanol 85-15) R_f : 0.47.

Analogous to examples 19-22, table 3 lists some further examples which were characterized as shown in table 4.

Table 3 Examples of 1-(2-deoxy-alpha/beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	alpha/beta	R ²
19	alpha	2-furyl
20	beta	2-furyl
21	alpha	2-thienyl
22	beta	2-thienyl
23	alpha	3-furyl
24	alpha	3-thienyl
25	beta	3-thienyl
26	alpha	2-selenenyl
27	beta	2-selenenyl
28	alpha	3-selenenyl
29	alpha	2-pyridyl
30	alpha	3-pyridyl
31	alpha	4-pyridyl
32	alpha	2-(5-methyl)thienyl
33	beta	2-(5-methyl)thienyl
34	alpha	2-(5-hexyl)thienyl
35	beta	2-(5-hexyl)thienyl
62	alpha	2-trans-cloften
63	beta	2-trans-cloften
64	alpha	2-cis-cloften
65	alpha	2-cis-cloften

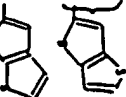


Table 4 Data for 1-(2-deoxy-alpha/beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	¹³ C NMR δ					¹ H NMR δ	Thin layer chromatography R_f
	1'	2'	3'	4'	5'	1'	
19	91.9	42.2	72.9	89.2	63.8 a	6.35 dd (J 2.9; 1 Hz) a	0.42 c
20	89.4	42.0	72.5	87.3	63.1 a	6.44 t (J 3.3 Hz) a	0.47 c
21	92.1	42.1	73.0	89.0	63.9 a	6.24 dd (J 2.9; 1.0 Hz) b	0.44 d
22	87.8	40.6	70.3	85.1	61.2 b	6.23 t (J 3.3 Hz) b	0.47 d
23	90.2	41.7	72.8	88.3	63.6 a	6.37 dd (J 3; 1 Hz) a	0.44 e
24							
25						6.36 t (J 5 Hz) a	
26							0.23 f
27							0.29 f
28						6.29 dd (J 5; 1 Hz) a	
29	91.7	42.2	72.8	89.5	63.7 a	6.33 dd (J 1.1; 1.5 Hz) a	0.43 g
30	92.1	42.1	73.0	89.1	63.9 a	6.30 d (J 3.2 Hz) a	
31	90.1		70.9	86.6	62.0 b	6.13 dd (J 3.0; 0.8 Hz) b	0.15 h
32	90.0	40.2	71.0	86.3	62.1 b	6.10 d (J 3.7 Hz) b	
33	87.8	40.5	70.3	84.9	61.2 b	6.23 t (J 3.4 Hz) b	
34	90.0	40.2	71.0	86.3	62.1 b	6.10 d (J 3.1 Hz)	
35	87.8	40.5	70.3	84.9	61.2 b	6.24 t (J 3.3 Hz)	

a) CD_3OD ; b) $DMSO-d_6$; c) $EtOAc-EtOH$ 18-1; d) $CHCl_3-MeOH$ 85-15; e) $EtOAc-EtOH$ 9-1

f) $CHCl_3-MeOH$ 7-1; g) $CHCl_3-MeOH$ 5-1; h) $EtOAc-MeOH$ 9-1

Table 4 Data for 1-(2-deoxy-alpha/beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	¹³ C NMR δ					¹ H NMR δ		Thin layer chromatography R _f	m.p. °C
	1'	2'	3'	4'	5'	1'			
4438									
62									140 (dec.)
63									242 (dec.)
64									227 (dec.)
65									240 (dec.)

24

90007448

Table 4 Additional data for 1-(2-deoxy-alpha/beta-D-ribofuranosyl)-5-R²-uracil compounds

Example	Elemental analysis		
	Calculated (found) %		
	C	H	N
25 x 1 H ₂ O	47.54 (47.7)	4.91 (4.5)	8.53 8.5
28 x 0.5 H ₂ O	42.63 (42.7)	4.13 (4.0)	7.65 (7.7)

25

4438

90007448

Example 36 1-(2,3-Dideoxy-5-O-ribofuranosyl)-5-(2-thienyl)uracil (VSB 523)

1-(2,3-Dideoxy-5-O-1-aryl-butylidiphenylsilyl-5-O-D-ribofuranosyl)-5-(2-thienyl)uracil (0.15 g) was dissolved in tetrahydrofuran, 1 M in tetrabutylammonium fluoride (3 ml) and stirred at ambient temperature for 1 hour. The solvent was evaporated and the product was purified by separation on preparative thin layer chromatography (silica, 10 mm, ethyl acetate-methanol 9-1) to give 1-(2,3-dideoxy-5-O-ribofuranosyl)-5-(2-thienyl)uracil. TLC (silica, ethyl acetate-methanol 9-1) Rf 0.54.

Example 37 1-(2,3-Dideoxy-5-O-ribofuranosyl)-5-(2-thienyl)uracil (VSB 534)

Starting from 1-(2,3-dideoxy-5-O-1-aryl-butylidiphenylsilyl-5-O-D-ribofuranosyl)-5-(2-thienyl)uracil (0.35 g) and using the same reaction conditions as described for the corresponding α -anomer in example 36, the title compound was obtained. TLC (silica, ethyl acetate-methanol 9-1) Rf 0.59.

The starting materials for the α - and β -anomers of 1-(2,3-dideoxy-5-O-ribofuranosyl)-5-(2-thienyl)uracil (examples 36 and 37 respectively) were prepared by the following sequence of reactions.

a) 5-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (VSB 526) and 5-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (VSB 527) were prepared by the following sequence of reactions. 5-(+)-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (25 g) was mixed with 80% acetic acid (aq, 400 ml) and stirred at 70-90°C for 2 hours. The solvent was evaporated in *vacuo* and the residue was chromatographed on a column of silica, eluted with ethyl acetate-hexane 1-2, to afford 5-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (VSB 525) as

90007448

4440

20-00000000

20-00000000

an oil (7.24 g, 90%). This product was dissolved in dry dimethyl formamide (600 ml), imidazole (10.6 g) followed by 1-aryl-butylidiphenylchlorosilane (25 ml, 25.7 g) were added and the solution was stirred at ambient temperature for 4 hours and then at 60°C for another hour. The solvent was evaporated in *vacuo*, the residue was dissolved in ethyl acetate, the solution was extracted with water and brine, dried (MgSO₄) and the solvent was evaporated in *vacuo*. The residue was purified by chromatography on a column of silica (ethyl acetate-hexane, 1-4) to yield 5-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (16.9 g, 77%, m.p. 76.5-77°C).

b) 2,3-Dideoxy-5-O-1-aryl-butylidiphenylsilyl-D-ribofuranose (VSB 527)

5-Y-1-aryl-butylidiphenylsilyloxymethyl- γ -butyrolactone (17.1 g) in dry diethyl ether (200 ml) was cooled to -78°C and stirred while diisobutylaluminumhydride in hexane (75 ml, 1.1 M) was added during 15-20 minutes. The stirring was continued for 1 hour at -78°C after which methanol (35 ml) was added and the reaction solution was allowed to come to room temperature. An aqueous sodium potassium tartrate solution (30%, 150 ml) was added with stirring. The organic phase was separated and extracted with the tartrate salt solution (4 x 75 ml). The combined aqueous portions were extracted with diethyl ether (4 x 75 ml). The combined organic solutions were dried (MgSO₄) and the solvent was evaporated to give 2,3-dideoxy-5-O-1-aryl-butylidiphenylsilyl-D-ribofuranose (16.3 g) as a viscous clear oil.

c) 1-Acetyl-2,3-dideoxy-5-O-1-aryl-butylidiphenylsilyl-D-ribofuranoside (VSB 528)

Acetic anhydride (15 ml) was added dropwise to an ice-cooled

90007448

4441

20-00000000

20-00000000

29

2-anomer: 0.10 g TLC (silica, ethyl acetate-hexane, 1-1) Rf 0.42. ¹³C NMR (CDCl₃): 26.20 (C3'); 26.98 (CH₃); 32.7 (C2'); 65.60 (C5'); 81.68 (C4'); 86.87 (C1'); 109.78 (C5); 125.40, 126.93, 127.2, 133.2 (thienyl); 127.76, 127.86, 129.98, 135.64 (phenyl); 133.6 (C6); 149.67 (C2); 162 (C4).

2-anomer: 0.38 g, TLC (silica, ethyl acetate-hexane, 1-1) Rf 0.60. ¹³C NMR (CDCl₃): 26 (C3'); 27 (CH₃); 33 (C2'); 66 (C5'); 82 (C4'); 88.5 (C1'); 125, 127, 133 (thienyl); 128, 130, 136 (phenyl); 134 (C6).

Example 28 1-(2,5,6-Trideoxy-α-D-ribo-hexofuranosyl)-6-phosphonic acid-5-(2-thienyl)uracil (VSB 823)

1-(2,5,6-Trideoxy-6-dimethylphosphono-α-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (214 mg) was heated at reflux in hexamethyl-disilazane (5 ml) and acetonitrile for about 15 minutes until all material was dissolved. The solvent was evaporated, bromotrimethylsilane (0.2 ml) in acetonitrile (5 ml) was added and the solution was stirred at ambient temperature for 3 hours. Aqueous ammonia (25%, 5 ml) was added, the solvent was evaporated and the residue was dissolved in water-dimethyl sulfoxide (about 1 ml). After filtration trifluoroacetic acid (10 drops) and acetone (5 ml) was added, the precipitate was collected and washed (decanted) with acetone (3x5 ml), to yield 1-(2,5,6-trideoxy-α-D-ribo-hexofuranosyl)-6-phosphonic acid-5-(2-thienyl)uracil. TLC (polyethylene imine, Macherey-Nagel, 0.2 M LiCl, molybdate spray-reagent) Rf 0.15.

Example 29 1-(2,5,6-Trideoxy-β-D-ribo-hexofuranosyl)-6-phosphonic acid-5-(2-thienyl)uracil (VSB 822)

Starting from 1-(2,5,6-trideoxy-6-dimethyl-phosphono-β-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (170 mg) and using the same reaction conditions as described for the corresponding α-anomer:

90007448

443

A solution of 2,3-dideoxy-5-O-isobutylidiphenylsilyl-D-ribofuranose (7.58 g) in dry pyridine (25 ml). The stirring was continued at room temperature for 14 hours after which the reaction solution was poured onto ice and extracted with diethyl ether. The ether solution was washed with water, followed by a saturated aqueous sodium hydrogencarbonate solution, water and brine and then dried (MgSO₄). The solvent was evaporated to give 1-acetyl-2,3-dideoxy-5-O-isobutylidiphenylsilyl-D-ribofuranoside as a slightly yellow oil (6.90 g, 89%).

d) 1-(2,3-Dideoxy-5-O-isobutylidiphenylsilyl)-α-D-ribofuranosyl)-5-(2-thienyl)uracil (VSB 530) and

e) 1-(2,3-Dideoxy-5-O-isobutylidiphenylsilyl)-β-D-ribofuranosyl)-5-(2-thienyl)uracil (VSB 529)

5-(2-Thienyl)uracil (0.65 g) was suspended in hexamethyldisilazane (30 ml) and chlorotrimethylsilane (0.5 ml) and heated at 90°C overnight together with a small amount of ammonium sulfate.

The solvent was evaporated in vacuum and the residual bis-trimethylsilylated 5-(2-thienyl)uracil was dissolved in dry acetonitrile (10 ml) together with 1-acetyl-2,3-dideoxy-5-O-isobutylidiphenylsilyl-D-ribofuranoside (1.75 g). The solution was cooled to -35°C and SnCl₄ (1.14 g, 0.51 ml) in dry acetonitrile (5 ml) was added dropwise. The reaction temperature was raised to -15°C and an excess of ammonia in methanol was added. The solution was allowed to reach room temperature, the solvent was evaporated in vacuum; the residue was extracted with ethyl acetate, filtered, the solvent was again evaporated in vacuum and the residue was subjected to chromatography on a column of silica eluted with ethyl acetate-hexane 1-9, to give the α- and β-anomers of 1-(2,3-dideoxy-5-O-isobutylidiphenylsilyl)-D-ribofuranosyl)-5-(2-thienyl)uracil.

90007448

442

(example 38), the title compound was obtained (40 mg). TLC (polyethylene imine, Macherey-Nagel, 0.2 M LiCl, molybdate spray reagent) Rf 0.15. ¹³C NMR (DMSO-d₆) δ: 22.70, 25.45 (C5'); 26.96 (C6'); 39.86 (C2'); 72.06 (C3'); 85.75 (C1'); 88.64, 88.98 (C4'); 108.10 (C5); 122.99, 126, 126.83, 134.55 (thienyl); 138 (C6); 149.82 (C2); 161.62 (C4).

The starting materials for the α- and β-anomers of 1-(2,5,6-trideoxy-2-tribg-hexofuranosyl-6-phosphonic acid)-5-(2-thienyl)-uracil (examples 38 and 39 respectively) were prepared by the following reaction sequence (a-h).

a) Methyl-2-deoxy-3-O-p-toluoyl-5-O-tert-butylidiphenylsilyl-D-ribofuranoside

Imidazole (18.9g) and tert-butylidiphenyl-chlorosilane (37.7 g) were added to methyl-2-deoxyribofuranoside (20.3 g) dissolved in dimethylformamid (150 ml) and the solution was stirred at ambient temperature over night. Thin layer chromatography (TLC, silica, ethyl acetate-hexane 1-4) shows the reaction product methyl-2-deoxy-5-O-tert-butylidiphenylsilyl-D-ribofuranoside with Rf 0.2. The solvent was evaporated in vacuo, the residue was dissolved in diethyl ether washed with water (4 x 50 ml), dried (MgSO₄) and the solvent was evaporated to give a residue (47.1g). The residue was dissolved in pyridine (200 ml), p-toluoylchloride (21.18 g) was added and the solution was stirred at ambient temperature for about 1 hour after which the solvent was evaporated in vacuo, the residue was taken up in diethyl ether and washed with water. The solution was dried (MgSO₄) and the solvent was evaporated in vacuo to give the title compound (50 g). TLC (silica, ethyl acetate-hexane 1-4) Rf 0.5. ¹³C NMR (CDCl₃) δ: 21.77 (CH₃, p-tol.); 26.73, 26.90 (CH₃, tert-but.); 39.41 (C2); 55.41 (OCH₃); 65.02 (C5); 75.05 (C3); 84.29 (C4); 105.77 (C1); 127.78, 128.34, 129.17, 129.00, 129.77, 134.96, 135.73 (C, phenyl).

90007448

b) Methyl-2-deoxy-3-O-p-toluoyl-D-ribofuranoside (VSB 818)

Methyl-2-deoxy-3-O-tert-butylidiphenylsilyl-D-ribofuranoside (50 g) was dissolved in a 1M solution of tert-butylammonium fluoride in tetrahydrofuran (100 ml). Dry sodium hydrogencarbonate (1 eq, 177 mmol) was added and the mixture was stirred at ambient temperature over night, after which the solvent was evaporated, the residue was washed with water and purified by chromatography on a silica column, eluted with ethylacetate-hexane (1-4), followed by ethyl acetate, to give the title compound (16.65 g). TLC (silica, ethyl acetate-hexane), 1-4) Rf 0.1. ¹³C NMR (CDCl₃) δ: 21.80 (CH₃, p-tol.); 40.14 (C2); 55.68 (OCH₃); 64.10 (C5); 75.97 (C3); 86.35 (C4); 105.84 (C1); 129.24, 129.70, 129.80, 129.93 (C, phenyl).

c) Diphenyl (1-methyl-2,5,6-trideoxy-3-O-p-toluoyl-D-ribo-hex-5-enofuranos-6-yl)-phosphonate (VSB 818)

Pyridinium trifluoroacetate (1.88 g) (prepared from equimolar amounts of pyridine and trifluoroacetic acid in diethyl ether) and some molecular sieves (4 Å) were added to a solution of methyl-2-deoxy-3-O-p-toluoyl-furanoside (5.26 g) in dimethylsulfoxide (40 ml). The solution was stirred at ambient temperature for about 30 minutes after which dicyclohexylcarbodiimide (12.2 g) was added and the stirring was continued at 60°C for 3 hours. TLC (silica, ethyl acetate-hexane, 1-4) shows a positive reaction with dinitrophenyl hydrazin-sulfuric acid spray at Rf 0.1. Methanol (20 ml) was added and stirring was continued at 50°C for another hour, after which methanol was evaporated in vacuo, the solution was filtered, diphenyl((triphenylphosphoryl)idene)methyl)phosphonate (9 g; G.W. Jones, E.K. Hamamura, J. Moffat, Tetrahedron Lett. (1968), 5371; J.A. Montgomery, A.G. Lasserer, K. Newton, J. Heterocyclic Chem. 11 (1974) 211) was added.

90007448

added and the solution was stirred at 70°C for 3 hours. After cooling, diethyl ether (200 ml) was added, the solution was washed with water (4 x 100 ml) and the ether solution was evaporated to dryness. The residue was purified by chromatography on a column of silica (500 g) eluted with ethyl acetate-hexane 1-4, yielding 4.4 g of diphenyl(1-methyl-2,5,6-trideoxy-3-O-p-toluenyl-D-ribo-hex-5-enofuranos-6-yl)phosphonate. ¹³C NMR (CDCl₃) δ: 21.70 (CH₃, p-tol); 37.85 (C2); 56.00 (OCH₃); 77.45 (C3); 83.78, 84.24 (C4); 106.52 (C1); 115.33, 119.12 (C5); 152.40, 152.52 (C6); 165.97 (CO).

d) Diphenyl(1-methyl-2,5,6-trideoxy-3-O-p-toluenyl-D-ribo-hexofuranos-6-yl)phosphonate (VSB 819).

Diphenyl(1-methyl-2,5,6-trideoxy-3-O-p-toluenyl-D-ribo-hex-5-enofuranos-6-yl)phosphonate (4.46 g) in dry tetrahydrofuran was hydrogenated at 1 bar for 30 minutes using Pd/C (5%) as a catalyst. The reaction mixture was filtered through a celite pad, the solvent was evaporated and the residue was purified by chromatography, on silica to give the title compound (3.72 g). ¹³C NMR (CDCl₃) δ: 21.53 (CH₃, p-tol); 21.21, 24.06 (C5); 27.68 (C6); 38.95 (C2); 55.27 (OCH₃); 77.52 (C3); 83.68, 84.04 (C4); 105.50 (C1); 166.02 (CO).

e) 1-(2,5,6-Trideoxy-3-O-p-toluenyl-6-diphenylphosphono-α-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (VSB 826) and

f) 1-(2,5,6-Trideoxy-3-O-p-toluenyl-6-diphenylphosphono-α-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (VSB 820)

5-(2-Thienyl)uracil (0.5 g) in dry acetonitrile (15 ml), hexamethyl diisilazane (5 ml) and chlorotrimethyl silane (0.5 ml) was heated at reflux for about 30 minutes after which the solvents were evaporated to give 2,4-bis-trimethyl silylated 5-(2-thienyl)uracil. Dry acetonitrile was added, followed by

diphenyl(1-methyl-2,5,6-trideoxy-3-O-p-toluenyl-D-ribo-hexofuranos-6-yl)phosphonate (VSB 819, 1.65 g) in dry acetonitrile (10 ml) and finally 1-tert-butyl-dimethylsilyltriflate (0.6 ml) under vigorous stirring, and the solution was stirred at ambient temperature for about 1.5 hours, after which concentrated aqueous ammonia (4 ml) was added. The solvent was evaporated in vacuo and the residue was purified by chromatography in a column of silica (100 g) eluted with ethyl acetate-hexane, 1-1, to give the α-anomer (0.56 g) and the β-anomer (0.40 g) of 1-(2,5,6-trideoxy-3-O-p-toluenyl-6-diphenyl-phosphono-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil. TLC (silica, ethyl acetate-hexane, 1-1) R_f: α 0.15; β 0.20. ¹³C NMR (CDCl₃) δ, α-anomer: 20.12 (CH₃, p-tol.); 19.68, 22.53 (C5); 25.40, 25.47 (C6); 36.81 (C2); 76.50 (C3); 85.84, 86.16 (C4); 86.35 (C1); 108.22 (C5); 122.89, 124.23, 125.49 (thienyl); 134.03 (C8), β-anomer: 21.80 (CH₃, p-tol.); 21.21, 24.08 (C5); 27.08 (C6); 37.27 (C2); 76.48 (C3); 84.12, 84.48 (C4); 85.70 (C1); 110.75 (C5); 124.76, 125.62, 127.17 (thienyl); 133.86 (C8).

g) 1-(2,5,6-Trideoxy-6-dimethylphosphono-α-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (VSB 825)

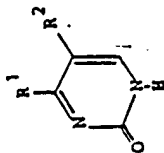
1-(2,5,6-Trideoxy-3-O-p-toluenyl-6-diphenylphosphono-α-D-ribo-hexofuranosyl)-5-(2-thienyl)uracil (444 mg) was dissolved in 0.5 M sodium methoxide in methanol (20 ml) and stirred at ambient temperature for 3 hours. The solution was neutralized with Dowex 50W x 8 (pyridinium⁺), filtered and the solvent was evaporated. Silica and diethyl ether-hexane was added, the solvent was decanted and the residue was again triturated with ether-hexane (4x). Finally the silica was eluted with methanol-tetrahydrofuran 1-1 and the solvent was evaporated in vacuo to give the title compound (234 mg). ¹³C NMR (CD₃OD) δ: 18.95, 21.80 (C5); 25.98, 26.08 (C6); 39.75 (C2); 52.49 (2 POCH₃); 73.32 (C3); 86.28 (C1); 88.25, 88.57 (C4); 108.29 (C5); 123.79; 125.10, 126.59, 133.68 (thienyl); 136.59 (C8); 149.02 (C2); 161.91 (C4).

34

h1-1-(2,5,6)-trideoxy-3-O-2-toluoyl-6-diphenyl-phosphono-β-D-ribo-hexo(uranosyl)-5-(2-thienyl)uracil (326 mg) and using essentially the same reaction conditions as described for the α-anomer, the title compound was obtained. In the work-up procedure for the β-anomer no silica was included; instead the crude product was dissolved in diethyl ether and by addition of hexane the product precipitated (190 mg). ¹³C NMR (CDCl₃-DMSO-d₆)δ: 18.90, 21.75 (C5'); 25.98, 26.08 (C6'); 39.43 (C2'); 52.08 (2 POCH₃); 73.05 (C3'); 85.38, 85.45, 85.80 (C1', C4'); 109.78 (C5); 123.86, 125.13, 126.52, 133.13 (thienyl); 134.57 (C6); 149.38 (C2); 162 (C4).

Starting from 1-(2,5,6-trideoxy-3-O-2-toluoyl-6-diphenyl-phosphono-β-D-ribo-hexo(uranosyl)-5-(2-thienyl)uracil (326 mg) and using essentially the same reaction conditions as described for the α-anomer, the title compound was obtained. In the work-up procedure for the β-anomer no silica was included; instead the crude product was dissolved in diethyl ether and by addition of hexane the product precipitated (190 mg). ¹³C NMR (CDCl₃-DMSO-d₆)δ: 18.90, 21.75 (C5'); 25.98, 26.08 (C6'); 39.43 (C2'); 52.08 (2 POCH₃); 73.05 (C3'); 85.38, 85.45, 85.80 (C1', C4'); 109.78 (C5); 123.86, 125.13, 126.52, 133.13 (thienyl); 134.57 (C6); 149.38 (C2); 162 (C4).

The precursor 5-substituted pyrimidine compounds of the formula I',

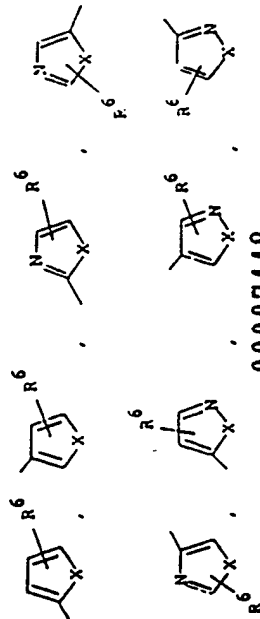


I'

wherein the radicals R¹ and R² are defined as follows:

R¹: OH, NH₂;

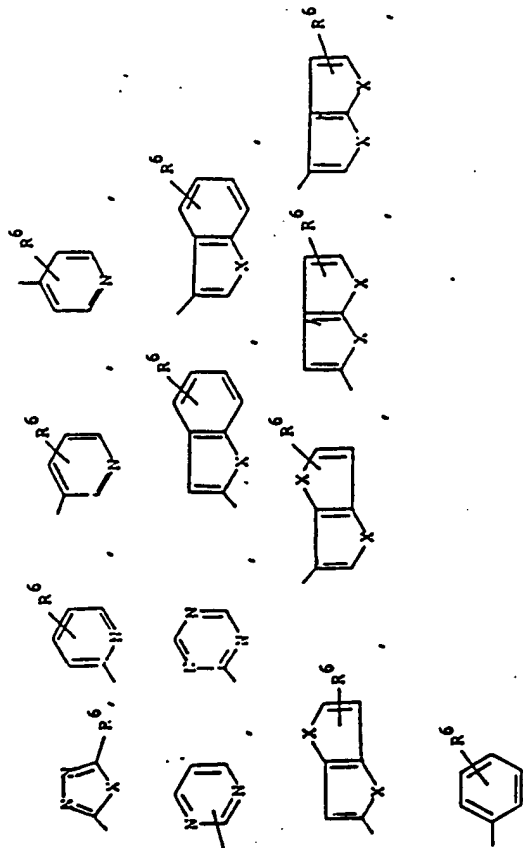
R²:



90007448

- 4148

35



wherein X is O, S, N-R⁷, Se;

R⁶ is H, straight or branched C₁₋₁₀ alkyl, F, Cl, Br, I,

X-R⁷, -CH=CH-R⁷, -C≡C-R⁷, CO₂R⁷, CH₂X-R⁷;

R⁷ is H, straight or branched C₁₋₅ alkyl, phenyl; constitute a further aspect of the invention.

The compounds of the formula I' may be prepared by the following general method:

The 2,4-dialkoxy-5-halopyrimidine compound may be reacted with the boronic acid or trialkylstannyl derivative of the heterocycle; alternatively the 2,4-dialkoxy-5-boronic acid pyrimidine or 2,4-dialkoxy-5-trialkylstannyl pyrimidine may be reacted with the halogen derivative of the heterocycle. In all cases the reaction is catalyzed by a palladium complex and performed in an organic solvent such as for example tetrahydrofuran or 1,2-dimethoxyethane at a temperature from

90007448

- 4149

A 250 ml flask equipped with condenser, magnetic stirrer and nitrogen inlet was charged with 7.3 g (0.024 mole) of 5-bromo-2,4-di-tert-butoxypyrimidine, 0.75 mmol of tetrakis(triphenylphosphine)palladium (0) and 60 ml of 1,2-dimethoxyethane. After stirring for 10 min 3.0 g (0.027 mole) of 3-furanboronic acid was added, immediately followed by 60 ml of 1M sodium carbonate solution. The reaction mixture was refluxed for 4 hours with vigorous stirring under nitrogen. After cooling to room temperature, the traces of catalyst were filtered off, the organic solvent was evaporated under reduced pressure and the residue diluted with water and extracted with three 50 ml portions of ether. The combined ethereal phases were washed with water, saturated sodium chloride solution and dried over magnesium sulphate. The ether was evaporated and the residue was purified by flash chromatography using hexane-ethyl acetate (4:1) as eluent. Yielding 4.1 g (59%) of the title compound as an oil.

Anal. Found C 66.5, H 7.66, N 9.64, O 17.0.

Calc. for $C_{16}H_{22}N_2O_3$ (290.4) C 66.2, H 7.64, N 9.65, O 16.5.

Example 42 5-[2'-(N-methyl)pyrrolyl]uracil

3.0 g (9.9 mmole) of 2,4-di-tert-butoxy-5-[2'-(N-methyl)pyrrolyl]pyrimidine was stirred with 40 ml of methanol and 40 ml of 5 M hydrochloric acid for 30 min. The precipitated crystals were collected by filtration, washed with methanol and water and dried, yielding 1.5 g (79%) of the title compound melting with decomposition over 250°C.

Anal. Found C 56.0, H 4.70, N 22.00.

Calc. for $C_9H_9N_3O_2$ (191.2): C 56.5, H 4.47, N 22.0.

The starting material 2,4-di-tert-butoxy-5-[2'-(N-methyl)pyrrolyl]pyrimidine was prepared as follows:

90007448

4453

90007448

A 250 ml flask equipped with condenser, magnetic stirrer and nitrogen inlet was charged with 9.0 g (29.7 mmole) 2,4-di-tert-butoxy-5-bromopyrimidine, 1.05 g (1.50 mmole) of $PdCl_2(PtC_6H_5)_2$ and 8.0 g (32.7 mmole) of N-methyl-2-trimethylstannylpyrrole in 80 ml of anhydrous tetrahydrofuran and refluxed for 20 hours. After cooling the reaction mixture, it was diluted with 200 ml of ether and washed twice with 50 ml of water. After drying with magnesium sulphate and evaporating the solvent, the compound was purified by chromatography using "silicagel 60" and a mixture of pentane-ether (9:1) as eluent, yielding 3.5 g (39%) of the title compound, mp 113-114°C.

Anal. Found C 67.0, H 8.37, N 13.7.

Calc. for $C_{17}H_{25}N_3O_2$ (303.4) C 67.3, H 8.30, N 13.8.

Analogous to example 40, Table 5 gives some further examples of preparations from 2,4-di-tert-butoxy-5-pyrimidine boronic acid and a bromo substituted heterocyclic compound. Their characteristics are given in table 6.

40

Table 5 Examples of 5-R²-uracil compounds

Example	R ²	Intermediate		5-R ² -uracil	
		2,4-di-tert-butoxy-5-(R ²)	yield %	mp °C	yield %
40	2-(5-chloro)thienyl		76	82-83.5	86
43	2-(5-methyl)thienyl		50	65-66	93
44	2-(5-hexyl)thienyl		52	0-11	90
45	2-furyl		49	87-88	100
46	2-thiazolyl		49	102-103	100
47	5-thiazolyl		62	68-69	100
48	2-pyridyl		60	128-129	100
49	3-pyridyl		69	88-89	100
50	4-pyridyl		70	92-93	100
51	2-methoxyphenyl		35	90-91.5	90
52	3-methoxyphenyl		65	93-94	90
53	4-methoxyphenyl		41	92-94	90
54	2,5-dimethoxyphenyl		47	91-93	100
66	2-trans-tloften		57	108-110	
67	2-cis-tloften		60	108-110	
68	3-trans-tloften		55	105-107	
69	3-cis-tloften		27	88-90	

Table 6 ¹H NMR chemical shifts (ppm, in DMSO-d₆) for 5-substituted uracil compounds

Example	H11	H12	H6	H2'	H3'	H4'	H5'	H6'	C11 ₂	OC11 ₂	OC11 ₂	C11 ₂
40	11.5	8.07			7.03	7.33	-	-				
43	9.3	7.83			7.22	6.71	-	-	2.51			2.73 a
44	11.39	11.20	7.83	-	7.24	6.73	-	-				
45	11.40	11.20	7.71	-	6.83	6.57	7.62	-				
46	11.85	11.78	8.55	-	-	7.92	7.71	-				
47	11.56	11.61	8.22	9.11	-	8.39	-	-				
48	11.90	11.74	8.49	-	8.67	8.20	7.58	8.33				
49	11.69	11.58	8.13	9.12	-	8.70	7.98	7.76				
50	12.00	11.66	8.43	8.77	8.37	-	8.37	8.77				
51	11.17	10.97	7.38		7.03	7.30	6.93	7.20		3.73		
52	11.20	7.65	7.13	-	6.86	7.26	7.13					
53	11.19	11.02	7.53	7.48	6.94	-	6.90	4.75		3.76		
54	11.17	10.95	7.39	-	6.95	6.85	-	6.85		3.70	3.67	

a) Additional bands at δ 1.64; 1.32; 0.88 with the relative intensities 2:6:3

4454

90007448

4455

90007448

Biological tests

Test 1 Effect of compounds of the formula I on HIV in H9 cells

Materials and methods: HIV infection of H9 cells

H9 cells, 10⁵ cells per well on a 24 well plate, suspended in 2 ml RPMI-medium containing 10% fetal calf serum, 100 µg/ml penicillin, 10 µg/ml streptomycin sulfate and 2 µg/ml polybrene are exposed to HIV (HTLV-III_B) and different concentrations of the test compounds. The plates are incubated at 37°C in 5% CO₂ for 6-7 days. The contents in each well is then homogenized with a pipette and transferred to a centrifuge tube. After centrifugation for 10 min at 1500 rpm the supernatant is removed and the cell pellet is analyzed by fixing in methanol on glass plates. Human HIV positive serum diluted 1:80 or 1:160 is added and incubated for 30 min at 37°C. The plate is then washed with phosphate-buffered saline (PBS) containing Ca²⁺ and Mg²⁺. Sheep antihuman conjugate (FITC) is added and after a new incubation the plate is again washed with PBS. Contrast staining is done with Evans blue and after drying the frequency of HIV antigen containing cells is determined in a microscope. The test result is shown in Table 7.

Table 7 Concentration (µM) for 50% inhibition (IC₅₀) of human immune deficiency virus multiplication in cell culture

1-(2'-deoxy-α/β-D-ribofuranosyl)-5-R²-uracil

R ²	Code	IC ₅₀ M
α 2-thienyl	VSA 134	0.05-10
α 2-selenienyl	VSA 188	2-20
α 3-selenienyl	VSA 996	3-100
α 2-furyl	VSF 007	<10
α 2-(5-methylthienyl)	VSF 515	10-10
β 3-selenienyl	VSA 992	5-10
β 2-thienyl	VSA 189	10-10
β 2-furyl	VSF 008	10-10

4456

90007448

Biological tests

Table 7 shows that the tested compounds are active inhibitors of HIV virus multiplication.

Test II Cellular toxicity

H9 cells, 2x10⁷ cells per plate, are incubated in RPMI-1640 medium containing 10% fetal calf serum, 70 mg/l penicillin, 100 mg/l streptomycin and 10 mM hepes, in absence or presence of test compounds. The number of cells per plate is determined after 48 h. Cells incubated in the absence of test compounds then underwent two cell division cycles.

F5000 cells, which are human embryo cells, 1x10⁵ cells per plate, are incubated in Eagle's minimal essential medium, supplemented with Earle's salts, non-essential amino acids, 10% fetal calf serum, 10 mM hepes, 70 mg/l penicillin and 100 mg/l streptomycin, in absence or presence of test compounds. The number of cells per plate is determined after 48 h. Cells incubated in the absence of test compounds underwent one cell division cycle. The results are given as % inhibition of cell multiplication when the concentration of the compound is 100 µM or 250 µM. The test results are given in table 8.

4457

90007448

Table 8 Cellular toxicity on H9 and F5000 cells

1-(2'-deoxy- α - β -D-ribofuranosyl)-5-P₂-uracil

g/g R ₂	Code	I inhibition (concentration μ M)	
		H9	F 5000
α 2-thienyl	VSA 134	35(250)	0-35(100)
α 2-selenienyl	VSA 188	40(200)	15(200)
α 3-selenienyl	VSA 996	65(200)	30(200)
α 2-furyl	VS8 007		
α 2-(5-methyl)thienyl	VS8 515		
β 3-selenienyl	VSA 992	40(200)	0(200)
β 2-thienyl	VSA 189	35(200)	10(100)
β 2-furyl	VS8 008		0(200)

Table 8 shows that the concentrations at which the compounds exhibit toxicities exceed the concentrations needed for 50% inhibition of HIV multiplication as given in table 7.

Test III Inhibition of reverse transcriptase and DNA polymerase by triphosphates of compounds of the invention

The 5'-triphosphates were synthesized essentially as described (Yoshikawa, M, Kato T, Takenishi T, Bull. Chem. Soc. (Japan), 42, 3505-3508, 1969; Ludwig, J., Acta Biochim. Biophys. Acad. Sci. Hung. 16, 131-133, 1981; Ruth, J.L., Cheng, Y.C., Mol. Pharmacol. 20, 415 1981.) The HIV-RT was obtained as described by Hansen et al (Hansen J, Schulze T and Moelling K, J. Biol. Chem. 262, 12393-12396, 1987) from cultures of *Escherichia coli* expressing the cloned HIV-pol gene. The HBV-DNAP was prepared from virus obtained from human serum, essentially as described by Nordenfelt et al (1987) (Nordenfelt E, Löfgren B, Chattopadhyaya J., Oberg B, J. Med. Virol. 22, 231-236, 1987). The HSV-2 DNAP and cellular DNAPs preparation and reaction conditions have been described by Larsson et al (Larsson A, Sundqvist A, Parnerud A-M, 1986, Mol. Pharmacol. 29, 614-621). In reactions using HIV-RT, the enzyme was incubated with

the template (rA)_n(dT)₁₂₋₁₈ and different concentrations of inhibitor and substrate (dNTP) as described by Vrang et al 1987, (Vrang L., Bazin M., Renaud G., Chattopadhyaya J. and Oberg B., Antiviral Res. 2, 139-145, 1987). The hepatitis B virus enzyme activity was determined with a virus preparation solubilized by non-idet P40, and endogenous nucleic acid as template, as described by Nordenfelt et al (vide supra)

Table 9 Concentration (μ M) for 50% inhibition (IC₅₀) of enzymes by triphosphates of some compounds of the invention

Compound	HIV RT1)	HBV DNAP2)	HSV-2 DNAP3)	DNAP4)
1-(2-Deoxy-beta-D-ribofuranosyl)-5-(2-thienyl)uracil-5'-triphosphate	0.015	0.11	0.06	1.6
1-(2-Deoxy-alpha-D-ribofuranosyl)-5-(2-thienyl)uracil-5'-triphosphate	2.0	18.0	11.0	80.0

- 1) Human immuno deficiency virus reverse transcriptase
- 2) Hepatit B virus DNA polymerase
- 3) Herpes simplex virus type 2 DNA polymerase
- 4) DNA polymerase alpha.

90007448

4459

90007448

4458

48

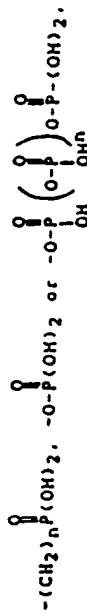
6. A compound according to any of claims 1-5, wherein R⁴ and R⁵ are both hydroxy.

7. A compound according to any of claims 1-5 wherein R³ and R⁴ are both hydrogen.

8. A compound according to any of claims 1-5 wherein R³ is hydrogen and R⁴ is fluoro, azido, cyano or methoxy.

9. A compound according to any of claims 1-5 wherein R³ is hydroxy and R⁴ is fluoro, azido, cyano or methoxy.

10. A compound according to any of claims 1-5, wherein R⁵ is



wherein n = 0 or 1

11. A compound according to any of claims 1-5, wherein R⁵ as an ether residue is defined as OR⁶, wherein R⁶ is C₁-6 alkyl, arylalkyl optionally substituted with one or more alkoxy, amino, nitrile or sulphamido groups or one or more halogen atoms.

12. A compound according to any of claims 1-5, wherein R⁴ and/or R⁵ as an ester residue is derived from a carboxylic acid R⁹COOH, a carbonic acid R¹⁰COOH, a double ester of a carbonic acid R¹⁰CO₂CH(R¹¹)CO₂H, a sulphonic acid R¹⁰SO₂OH, a carbamic acid R¹⁰NHCOOH or a phosphoric acid, wherein R⁹ is hydrogen, C₁-17 alkyl, alkoxyalkyl, arylalkyl or aryl, R¹⁰ is C₁-17 alkyl, arylalkyl or aryl, R¹¹ is hydrogen or C₁-3 alkyl and said aryl and arylalkyl groups optionally can be substituted with one or more alkyl, alkoxy, amino, nitrile, sulphonamido groups or one or more halogen atoms.

4462

90007448

49

13. A compound according to any of claims 1-12, wherein R² is 2-thienyl, 2-selenienyl, 2-furyl, 2-thiazolyl or 2-(1-methyl)-pyrrolyl or methoxyphenyl.

14. A compound of the formula I according to any of claims 1-13 for use in therapy.

15. A pharmaceutical composition comprising as an active ingredient a compound of the formula I according to any of claims 1-13 and a pharmaceutically acceptable carrier, including liposomes.

16. A method for therapeutic and/or prophylactic treatment of virus infections in an animal or human host in need of treatment, comprising administering an effective amount of a compound of the formula I as defined in any of claims 1-13.

17. A method according to claim 16 for treatment of infections caused by viruses requiring reverse transcriptase for replication, including human immuno deficiency virus and hepatitis B virus.

18. A method for treatment of aids comprising administering an effective amount of a compound of the formula I as defined in any of claims 1-13.

19. A method according to claim 16 for treatment of infections caused by herpes viruses.

20. Use of a compound of the formula I according to any of claims 1-13 for the manufacture of a medicament for therapeutic and/or prophylactic treatment of the acquired immuno deficiency syndrome and infections caused by viruses requiring reverse transcriptase for replication.

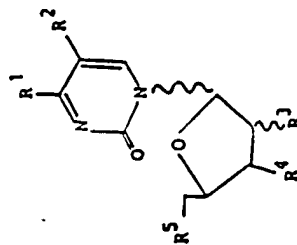
21. Use according to claim 20 for the treatment of infections caused by HIV-viruses.

4463

90007448

CLAIMS

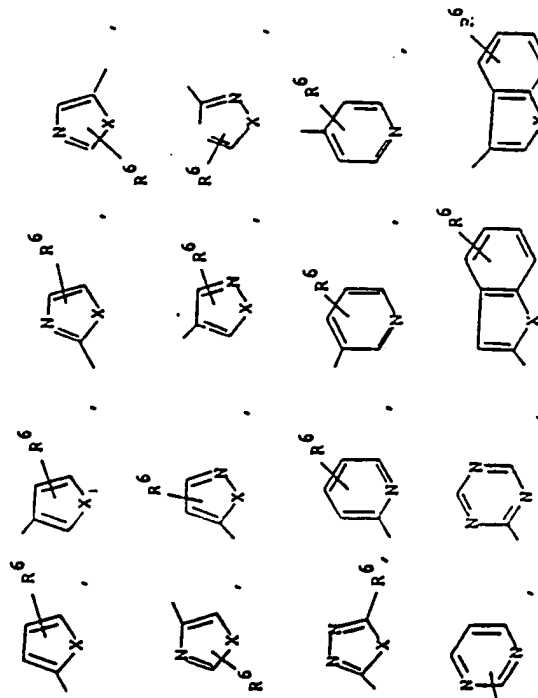
1. A compound of the formula



wherein the radicals R¹, R², R³, R⁴ and R⁵ are defined as follows:

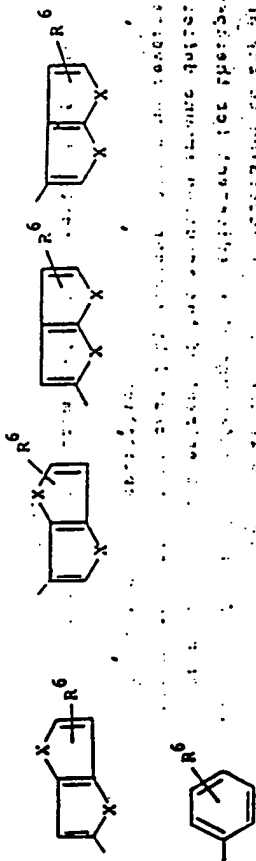
R¹: OH, NH₂;

R²:



4400

90007448



wherein X is O, S, N-R⁷, Se;

R⁶ is H, straight or branched C₁-10 alkyl, F, Cl, Br, I,

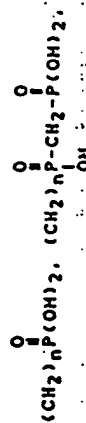
X-R⁷, -CH=CH-R⁷, -C≡C-R⁷, CO₂R⁷, CH₂X-R⁷;

R⁷ is H, straight or branched C₁-5 alkyl, phenyl;

R³: H, OH, F, OCH₃;

R⁴: H, F, OH or an ether or ester residue thereof, OCH₃, CH₃, C₂H₅, N₃;

N⁵: OH or an ether or ester residue thereof;



wherein n is 0 or 1 and H is hydrogen or a pharmaceutically acceptable counterion such as sodium, potassium, ammonium or alkylammonium; and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1 in the form of an alpha anomer.

3. A compound according to claim 1 in the form of a beta anomer.

4. A compound according to claim 2 or 3, wherein the carbohydrate moiety has the arabinofuranosyl configuration.

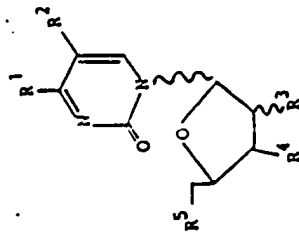
5. A compound according to claim 2 or 3, wherein the carbohydrate moiety has the ribofuranosyl configuration.

4481

90007448

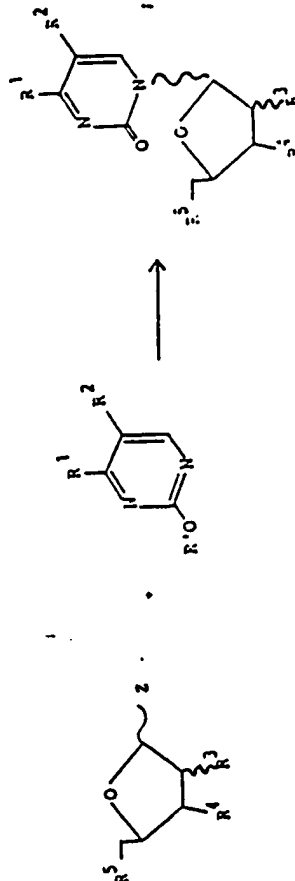
22. Use according to claim 20 for the treatment of infections caused by hepatitis B virus.

23. A process for preparation of a compound of the formula



wherein R¹, R², R³, R⁴ and R⁵ are as defined in claim 1, by

A. condensing a glycoside as comprised in formula I to the N-1 position of a pyrimidine derivative

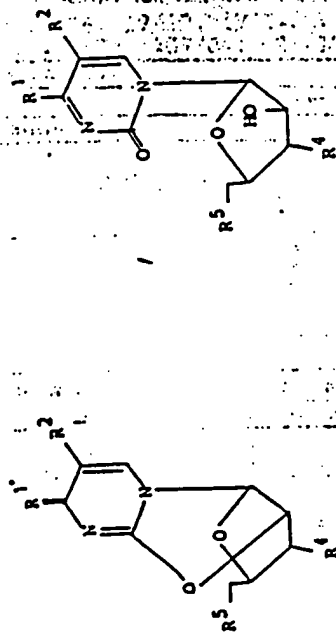


wherein Z is Cl, Br, I, acyloxy or alkoxy

4464

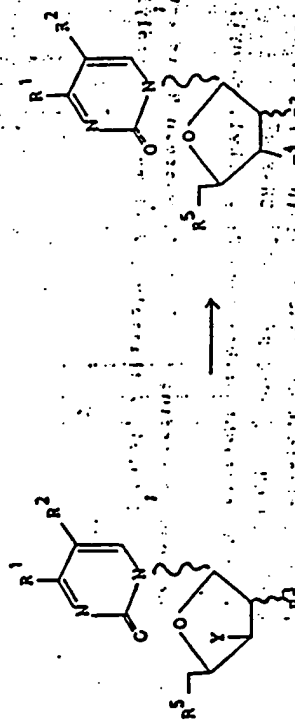
90007448

B. hydrolyzing a 2,2'-anhydro nucleoside analogue to the corresponding β-anomer of the arabinosyl-pyrimidine nucleoside analogue



wherein R¹ is O or NH;

C. substituting a derivatized hydroxyl group Y in the 3'-position of the glycoside moiety by a fluoro, OCH₃, N₃, CN or CsCH substituent

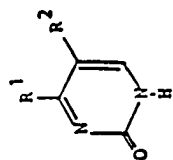


in which processes R¹-R⁵ and n are as defined in claim 1 and optionally may be protected by suitable protecting groups.

4485

90007448

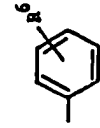
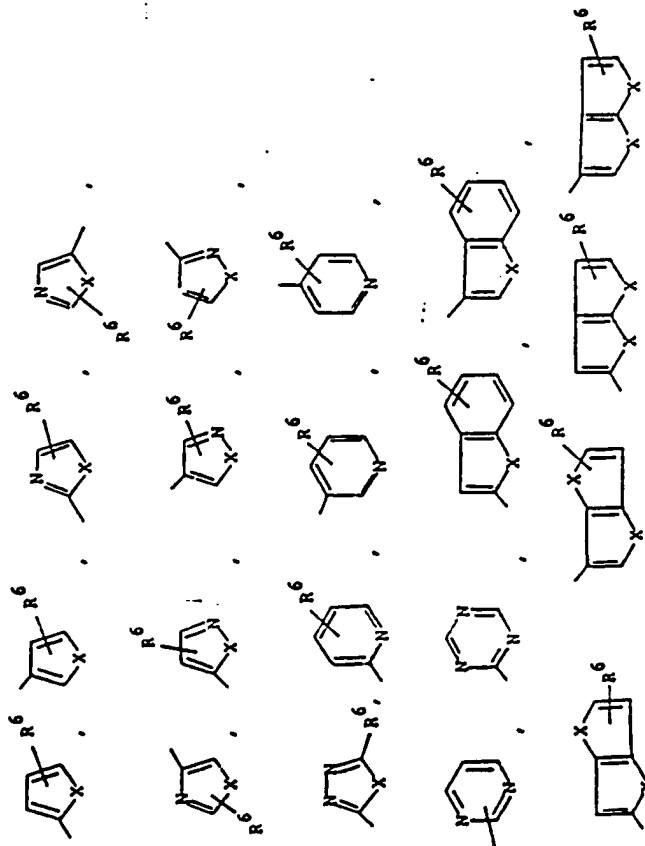
24. A compound of the formula



wherein the radicals R¹ and R² are defined as follows:

R¹: OH, NH₂;

R²:



90007448

wherein X is O, S, N-R⁷, Se;

R⁶ is H, straight or branched C₁-10 alkyl, F, Cl, Br, I, X-R⁷, -CH=CH-R⁷, -C≡C-R⁷, CO₂R⁷, CH₂X-R⁷;

R⁷ is H, straight or branched C₁-5 alkyl, phenyl;

25. A compound according to claim 24, wherein R² is 2-thienyl, 2-selenienyl, 2-furyl, 2-thiazolyl, 2-(1-methyl)pyrrolyl or methoxyphenyl.

4467

90007448

INTERNATIONAL SEARCH REPORT

990007448 International Application No. PCT/SE89/00322

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all.) According to International Patent Classification (IPC) or to both National Classification and IPC 4 C 07 H 19/06, 19/10, 19/24, A 61 K 31/70, C 07 D 239/47, 239/54, 401/04, 403/04, 405/04, 409/04, 421/04		
II. FIELDS SEARCHED Minimum Documentation Searched ? Classification System Classification Symbols IPC 4 C 07 H Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ? SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No. 10
X	Anales de la real Academia de farmacia, vol. 50, 1984 (1984):1, pages 57-65, Mohamed E. Hassan: "Photochemical synthesis of C-5 heteroaryl-substituted pyrimidine nucleosides".	1-15, 20-22
X	Tetrahedron Letters, Vol. 25, No. 2, pages 201-202, 1984, P. Vincent et al: "Synthese de Nucleosides substitues en C-5 par un carbocycle ou un heterocycle par couplages d'organozinciques avec L'1000-5 O-bis(trimethylsilyl)-3',5' desoxy-2' uridine catalyses par des complexes organo-palladiques".	1-15, 23
X	Chemical Abstracts, vol. 104, No. 23, 9 June 1986, (Columbus, Ohio, US), Hassan Mohamed E. "Photochemical synthesis of C(5)-alkyl and -heteroaryl-substituted pyrimidine nucleotides", see page 790, abstract 207587h, & Collect. Czech. Chem. Commun. 1985, 50(10), 2319-27 (Eng).	1,3,5,10,12,13
.../...		
* Special categories of cited documents: ** "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 1989-09-05		Date of Mailing of this International Search Report 1989-09-13
International Searching Authority Swedish Patent Office		Signature of Authorizing Officer Gunilla Claesson

Form PCT/ISA/210 (second sheet) (January 1988)

4468

90007448

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 98, no. 24, 13 June 1983, (Columbus, Ohio, US), Balzarini J. et al: "Structure-function relationship of the antitumor cell activity of pyrimidine and pyridine derivatives", see page 17 abstract 209571e, & Proc. Int. Round Table Nucleosides, Nucleotides Their Biol. Appl. 4th 1981 (Pub. 1982), 275-91. --	1-15, 20-22
X	Tetrahedron Letters, vol. 21, 1980, pages 2813-2816, Isao Saito et al: "A simple synthesis of fluorescent uridines by photochemical method".	1,3,5,6
X	Tetrahedron Letters, vol. 25, no. 23, 1984, pages 2431-2434, George Chang et al: "Palladium(0) Catalyzed Coupling Reactions in the Synthesis of 5-Arylpyrimidine Nucleosides".	1,3,5,6,13
X	J. Med. Chem. 1988, vol. 31, pages 1141-1147, George Chang et al: "Linear Free Energy Relationship Studies of Enzyme Active Site Binding: Thymidylate Synthase".	1-15, 20, 23
X	Chemical Abstracts, vol. 98, no. 7, 14 February 1983, (Columbus, Ohio, US), Budesinsky Zdenek et al: "Some 1- β -D-ribo-furanosyl-5-phenylcytosines and -5-(2-chlorophenyl)-2-thiocytosine", page 427, abstract 4734p, & Collect. Czech. Chem. Commun. 1982, 47(8), 2145-9 (Eng).	1-6, 23
X	US, A, 4 182 859 (SIEGFRIED ERHARDT) 8 January 1980 & DE, 2721466 JP, 53144586	1-15, 20-23
X	US, A, 4 211 773 (CARLOS LOPEZ ET AL.) 8 July 1980 & EP, 0010205 JP, 55049395 CA, 1131626 AU, 533135	1-15, 20-23
X	US, A, 4 666 892 (JACK J. FOX ET AL.) 19 May 1987 JP, 60252418 AU, 575918	1-15, 20-22
P,X	WO, A1, 8804662 (ASTRA LÄKEMEDEL AB) 30 June 1988 EP, 0294443	1-15, 20-22

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 98, no. 24, 13 June 1983, (Columbus, Ohio, US), Balzarini J. et al: "Structure-function relationship of the antitumor cell activity of pyrimidine and pyridine derivatives", see page 17 abstract 209571e, & Proc. Int. Round Table Nucleosides, Nucleotides Their Biol. Appl. 4th 1981 (Pub. 1982), 275-91.	1-15, 20-22
X	Tetrahedron Letters, vol. 21, 1980, pages 2813-2816, Isao Saito et al: "A simple synthesis of fluorescent uridines by photochemical method".	1,3,5,6
X	Tetrahedron Letters, vol. 25, no. 23, 1984, pages 2431-2434, George Chang et al: "Palladium(0) Catalyzed Coupling Reactions in the Synthesis of 5-Arylpyrimidine Nucleosides".	1,3,5,6,13
X	J. Med. Chem. 1988, vol. 31, pages 1141-1147, George Chang et al: "Linear Free Energy Relationship Studies of Enzyme Active Site Binding: Thymidylate Synthase".	1-15, 20, 23
X	Chemical Abstracts, vol. 98, no. 7, 14 February 1983, (Columbus, Ohio, US), Budesinsky Zdenek et al: "Some 1- β -D-ribo-furanosyl-5-phenylcytosines and -5-(2-chlorophenyl)-2-thiocyto-sine", page 427, abstract 4734p, & Collect. Czech. Chem. Commun. 1982, 47(8), 2145-9 (Eng).	1-6, 23
X	US, A, 4 182 859 (SIEGFRIED ERHARDT) 8 January 1980 & DE, 2721466 JP, 53144586	1-15, 20-23
X	US, A, 4 211 773 (CARLOS LOPEZ ET AL.) 8 July 1980 & EP, 0010205 JP, 55049395 CA, 1131626 AU, 533135	1-15, 20-23
X	US, A, 4 666 892 (JACK J. FOX ET AL.) 19 May 1987 JP, 60252418 AU, 575918	1-15, 20-22
P,X	WO, A1, 8804662 (ASTRA LÄKEMEDEL AB) 30 June 1988 EP, 0294443	1-15, 20-22

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 98, no. 24, 13 June 1983, (Columbus, Ohio, US), Balzarini J. et al: "Structure-function relationship of the antitumor cell activity of pyrimidine and pyridine derivatives", see page 17 abstract 209571e, & Proc. Int. Round Table Nucleosides, Nucleotides Their Biol. Appl. 4th 1981 (Pub. 1982), 275-91.	1-15, 20-22
X	Tetrahedron Letters, vol. 21, 1980, pages 2813-2816, Isao Saito et al: "A simple synthesis of fluorescent uridines by photochemical method".	1,3,5,6
X	Tetrahedron Letters, vol. 25, no. 23, 1984, pages 2431-2434, George Chang et al: "Palladium(0) Catalyzed Coupling Reactions in the Synthesis of 5-Arylpyrimidine Nucleosides".	1,3,5,6,13
X	J. Med. Chem. 1988, vol. 31, pages 1141-1147, George Chang et al: "Linear Free Energy Relationship Studies of Enzyme Active Site Binding: Thymidylate Synthase".	1-15, 20, 23
X	Chemical Abstracts, vol. 98, no. 7, 14 February 1983, (Columbus, Ohio, US), Budesinsky Zdenek et al: "Some 1- β -D-ribo-furanosyl-5-phenylcytosines and -5-(2-chloro-phenyl)-2-thiocytosine", page 427, abstract 4734p, & Collect. Czech. Chem. Commun. 1982, 47(8), 2145-9 (Eng).	1-6, 23
X	US, A, 4 182 859 (SIEGFRIED ERHARDT) 8 January 1980 & DE, 2721466 JP, 53144586	1-15, 20-23
X	US, A, 4 211 773 (CARLOS LOPEZ ET AL.) 8 July 1980 & EP, 0010205 JP, 55049395 CA, 1131626 AU, 533135	1-15, 20-23
X	US, A, 4 666 892 (JACK J. FOX ET AL.) 19 May 1987 JP, 60252418 AU, 575918	1-15, 20-22
P,X	WO, A1, 8804662 (ASTRA LÄKEMEDEL AB) 30 June 1988 EP, 0294443	1-15, 20-22